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Dated: August 2, 2012 Signature: (Shannon Reaney)

Docket No.: 146392015800
Client Ref. No.: P1467R2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Mark SLIWKOWSKI

Patent No.: 6,949,245

Issued: September 27, 2005

Application No: 09/602,812

For: HUMANIZED ANTI-ERBB2 ANTIBODIES AND TREATMENT WITH ANTI-ERBB2 ANTIBODIES – Application for § 156 Patent Term Extension

Attorney Docket No: 146392015800

Assignee: Genentech, Inc.

Unit: Office of Patent Legal Administration

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Dear Madam:

Applicant, Genentech, Inc., hereby submits this application for extension of the term of United States Letters Patent 6,949,245 under 35 U.S.C. § 156 by providing the following information in accordance with the requirements specified in 37 C.F.R. § 1.740.

Applicant represents that it is the assignee of the entire interest in and to United States Letters Patent No. 6,949,245, granted to Mark Sliwkowski by virtue of an assignment of such patent to Genentech, Inc., recorded August 3, 2000, at Reel 011128, Frame 0088.

10/10/2012 CKHLOK 00000015 031952 6949245
01 FC:1457 1120.00 DA

pa-1543975

1. Identification of the Approved Product [§ 1.740(a)(1)]

The name of the approved product is PERJETA™. The name of the active ingredient of PERJETA™ is pertuzumab. Pertuzumab is a recombinant humanized monoclonal antibody that targets the extracellular dimerization domain (Subdomain II) of the human epidermal growth factor receptor 2 protein (HER2[1]).

2. Federal Statute Governing Regulatory Approval of the Approved Product [§ 1.740(a)(2)]

The approved product was subject to regulatory review under, *inter alia*, the Public Health Service Act (42 U.S.C. § 201 *et seq.*) and the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355 *et seq.*).

3. Date of Approval for Commercial Marketing [§ 1.740(a)(3)]

PERJETA™ was approved for commercial marketing or use under § 351 of the Public Health Service Act on **June 8, 2012**.

4. Identification of Active Ingredient and Certifications Related to Commercial Marketing of Approved Product [§ 1.740(a)(4)]

(a) The active ingredient of PERJETA™ is pertuzumab. Pertuzumab is a recombinant humanized monoclonal antibody that targets the extracellular dimerization domain (Subdomain II) of the human epidermal growth factor receptor 2 protein (HER2).

(b) Applicant certifies that pertuzumab had not been approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act or the Virus-Serum-Toxin Act prior to the approval granted on June 8, 2012 to the present Applicant.

(c) Pertuzumab has been approved for use in the treatment of patients with HER2-positive metastatic breast cancer. *See* pertuzumab product label, provided as Attachment A.

(d) Pertuzumab was approved for commercial marketing pursuant to § 351 of the Public Health Service Act (42 U.S.C. § 262) under Genentech's existing Department of Health and Human Services (DHHS) U.S. License No. 1048 *See* pertuzumab approval letter, provided as Attachment B.

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[1] HER2 is synonymous with "ErbB2." *See* U.S. Patent No. 6,949,245, Attachment C, column 7, lines 43-44.

5. Statement Regarding Timeliness of Submission of Patent Term Extension Request [§ 1.740(a)(5)]

Applicant certifies that this application for patent term extension is being timely submitted within the sixty (60) day period permitted for submission specified in 35 U.S.C. § 156(d)(1) and 37 C.F.R. § 1.720(f). The last date on which this application may be submitted is August 6, 2012.

6. Complete Identification of the Patent for Which Extension Is Being Sought [§ 1.740(a)(6)]

The complete identification of the patent for which an extension is being sought is as follows:

(a) Name of the inventor: Mark Sliwkowski

(b) Patent Number: 6,949,245 ("the '245 patent")

(c) Date of Issue: September 27, 2005

(d) Date of Expiration: May 24, 2021[2]

7. Copy of the Patent for Which an Extension Is Being Sought [§ 1.740(a)(7)]

A copy of U.S. Patent No. 6,949,245 is provided as Attachment C to the present application.

8. Copies of Disclaimers, Certificates of Correction, Receipt of Maintenance Fee Payment, or Reexamination Certificate [§ 1.740(a)(8)]

(a) U.S. Patent No. 6,949,245 is not subject to a terminal disclaimer.

(b) A Certificate of Correction was issued for U.S. Patent No. 6,949,245 on December 6, 2005. A copy of the Certificate of Correction is provided in Attachment D to the present application.[3]

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[2] The face page of the '245 patent states that the patent term is extended or adjusted under 35 U.S.C. 154(b) by 215 days. A Petition Decision dated February 15, 2006 granted Applicant's request to correct the number of days provided under 35 U.S.C. 154(b) to 335 days and states that the application is being forwarded to the Certificates of Correction Branch for issuance of a Certificate of Correction to rectify the error. *See* Attachment K. A draft Certificate of Correction accompanied the Petition Decision. *Id.* A Certificate of Correction to correct this error has not issued to date; however, Applicant is to contact the U.S. Patent and Trademark Office to request its publication and has relied on the 335 days as stated in the Petition Decision to calculate the patent expiration date shown.

[3] *See* footnote 2.

(c) U.S. Patent No. 6,949,245 issued September 27, 2005. The first maintenance fee was paid February 25, 2009. *See* Attachment E. The window for paying the second maintenance fee on U.S. Patent No. 6,949,245 opens September 27, 2012 (*See* Attachment E). Therefore, no maintenance fee is currently due for U.S. Patent No. 6,949,245.

(d) U.S. Patent No. 6,949,245 has not been the subject of a reexamination proceeding.

9. Statement Regarding Patent Claims Relative to Approved Product [§ 1.740(a)(9)]

The statements below are made solely to comply with the requirements of 37 C.F.R. § 1.740(a)(9). Applicant notes that, as the M.P.E.P. acknowledges, § 1.740(a)(9) does not require an applicant to show whether or how the listed claims would be infringed, and that this question cannot be answered without specific knowledge concerning acts performed by third parties. As such, these comments are not an assertion or an admission of Applicant as to the scope of the listed claims, or whether or how any of the listed claims would be infringed, literally or under the doctrine of equivalents, by the manufacture, use, sale, offer for sale or the importation of any product.

(a) At least claims 1-6, 11, 14, 17, 19 and 24 of U.S. Patent No. 6,949,245 claim the active pharmaceutical ingredient in the approved product or the approved product or a method that may be used to make or use that ingredient or product.

(b) Pursuant to M.P.E.P. § 2753 and 37 C.F.R. § 1.740(a)(9), the following explanation is provided which shows how at least one of the above-listed claims of the '245 patent claim the approved product.

(1) Description of the approved product

The name of the approved product is PERJETA™. The name of the active ingredient of PERJETA™ is pertuzumab. Pertuzumab is described in Section 11 of the approved label for PERJETA™, a copy of which is provided as Attachment A, as a recombinant humanized monoclonal antibody that targets the extracellular dimerization domain (Subdomain II) of the human epidermal growth factor receptor 2 protein (HER2).

(2) Explanation Regarding Claim 24 of the '245 Patent Relative to Pertuzumab

Claim 24 of the '245 patent reads:

"24. A method of treating cancer in a human, wherein the cancer expresses epidermal growth factor receptor (EGFR) and ErbB2, comprising administering to the human a therapeutically effective amount of a humanized form of monoclonal antibody 2C4 (ATCC HB-12697)."

Pertuzumab is approved for use in the treatment of patients with HER2-positive metastatic breast cancer. *See* Attachment A. ErbB2 is synonymous with HER2 (*see* Attachment C, col. 7, lines 43-44). Since the cancer for which pertuzumab is indicated for use is HER2-positive, it therefore expresses HER2/ErbB2. In addition, since breast cancer is a type of cancer which expresses EGFR (*see* Attachment C column 46, lines 13, 14 and 23), the cancer for which pertuzumab is indicated for use also expresses EGFR. As such, pertuzumab is approved for use in a method of treating cancer in a human, wherein the cancer expresses EGFR and ErbB2. Pertuzumab is administered to a human in a therapeutically effective amount to treat the HER2-positive metastatic breast cancer. *See* Attachment A.

Pertuzumab is a humanized form of monoclonal antibody 2C4. An example of a humanized form of monoclonal antibody 2C4 is "humanized 2C4 version 574" whose variable domain amino acid sequences are depicted in Figs. 7A-B of the '245 patent. *See* Attachment C. The variable light and variable heavy amino acid sequences of pertuzumab comprise the amino acid sequences of Figs. 7A-B of the '245 patent (SEQ ID No. 3 and SEQ ID No. 4, respectively). *See* Attachment C, columns 67-68 and Attachment F.

The approved use of pertuzumab thus meets the limitations of claim 24.

10. Relevant Dates Under 35 U.S.C. § 156 for Determination of Applicable Regulatory Review Period [§ 1.740(a)(10)]

(a) Patent Issue Date

U.S. Patent No. 6,949,245 was issued on September 27, 2005.

(b) IND Effective Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(A)]

The date that an exemption under § 505(i) of the Federal Food, Drug and Cosmetic Act became effective (*i.e.*, the date that an investigational new drug application ("IND") became effective) for pertuzumab was September 11, 2001. The IND was assigned number BB-IND 9900. A copy of the letter from the FDA reflecting the effective date of the IND is provided in Attachment G. The application date for this IND was June 29, 2001.

(c) BLA Submission Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(B)]

The BLA was submitted by Genentech to the FDA on December 6, 2011. The BLA was assigned number 125409/0. A copy of the letter from the FDA acknowledging receipt of the BLA and reflecting the BLA submission date is provided in Attachment H.

(d) BLA Issue Date [35 U.S.C. § 156(g)(1)(B)(ii); 37 C.F.R. § 1.740(a)(10)(i)(C)]

The FDA approved BLA 125409/0 authorizing the marketing of pertuzumab on June 8, 2012. Pertuzumab was approved under Department of Health and Human Services (DHHS) U.S. License No. 1048. A copy of the approval letter from the FDA is provided as Attachment B.

11. Summary of Significant Events During Regulatory Review Period [§ 1.740(a)(11)]

Pursuant to 37 C.F.R. § 1.740(a)(11), the following provides a brief description of the activities of Genentech, Inc. before the FDA in relation to the regulatory review of pertuzumab. The brief description lists significant events that occurred during the regulatory review period for the approved product. In several instances, communications to or from the FDA are referenced. Pursuant to 37 C.F.R. § 1.740(a)(11), 21 C.F.R. § 60.20(a), and M.P.E.P. § 2753, copies of all such communications are not provided in this application, but can be obtained from records maintained by the FDA.

- On June 29, 2001, Genentech submitted to the FDA an Investigational New Drug (IND) application for recombinant humanized monoclonal antibody 2C4 (rhuMAb 2C4) (*See* Attachment I). The antibody was developed as a potential new therapeutic in treating patients with cancer. On September 11, 2001, the FDA made BB-IND 9900 effective via a communication received at Genentech on September 24, 2001 (*See* Attachment G).

- The first human clinical trial (Phase I) was initiated on November 8, 2001 followed by Phase II human trials and Phase III human trials (14 clinical studies), some of which remain ongoing at the time of this application.

- On March 20, 2003, Genentech submitted new protocol TOC2572g.

- On June 4, 2003, Genentech submitted new protocol TOC2689g.

- On July 9, 2003, Genentech submitted new protocol TOC2682g.

- On May 6, 2004, Genentech submitted new protocol TOC2664.

- On October 6, 2004, Genentech submitted new protocol TOC3258g.

- On April 17, 2007 representatives of Genentech and the FDA (CDER) participated in a Type B End-of-Phase II meeting.

- On November 5, 2007, a follow up Type C meeting was conducted to agree on the design of the pivotal Phase III trial.

- A phase III Randomized, Double-Blind, Placebo-Controlled Registration Trial to Evaluate the Efficacy and Safety of Placebo + Trastuzumab + Docetaxel versus Pertuzumab + Trastuzumab + Docetaxel in Patients with Previously Untreated HER2-Positive Metastatic Breast Cancer (CLEOPATRA) was the basis of the approval of pertuzumab.

- On October 22, 2008 and November 5, 2008, Genentech submitted new protocol TOC4603g.

- On May 24, 2011 and September 30, 2011 representatives of Genentech and CDER participated in Type C and Type B Pre-BLA submission meetings, respectively, to discuss information requirements for the BLA and the acceptability of the Phase III trial results to serve as the basis for the BLA.

- Genentech submitted a BLA for pertuzumab for the treatment of HER2-positive Metastatic Breast Cancer (MBA) on December 6, 2011.

- FDA acknowledged receipt of the BLA for pertuzumab via a communication mailed to Genentech dated December 21, 2011. The letter indicated that FDA had assigned the Submission Tracking Number (STN) of BLA 125409/0 to the BLA (*See* Attachment H).

- On June 8, 2012 FDA approved BLA 125409/0, issuing marketing authorization for pertuzumab (*See* Attachment B).

12. Statement Concerning Eligibility for and Duration of Extension Sought Under 35 U.S.C. § 156 [37 C.F.R. § 1.740(a)(12)]

(a) In the opinion of the Applicant, U.S. Patent No. 6,949,245 is eligible for an extension under § 156 because:

(i) one or more claims of the '245 patent claim the approved product or a method of making or using the approved product;

(ii) the term of the '245 patent has not been previously extended on the basis of § 156;

(iii) the '245 patent has not expired;

(iv) no other patent has been extended pursuant to § 156 on the basis of the regulatory review process associated with the approved product;

(v) there is an eligible period of regulatory review by which the patent may be extended pursuant to § 156;

(vi) the applicant for marketing approval exercised due diligence within the meaning of § 156(d)(3) during the period of regulatory review;

(vii) the present application has been submitted within the 60-day period following the approval date of the approved product, pursuant to § 156(c); and

(viii) this application otherwise complies with all requirements of 35 U.S.C. § 156 and applicable rules and procedures.

(b) The period by which the term of the '245 patent is requested by Applicant to be extended is **1,317 days**.

(c) The requested period of extension of term for the '245 patent corresponds to the regulatory review period that is eligible for extension pursuant to § 156, based on the facts and circumstances of the regulatory review associated with the approved product and the issuance of the '245 patent. The period was determined as follows.

(i) The relevant dates for calculating the regulatory review period, based on the events discussed in the section above, are the following:

Exemption under FDCA § 505(i) became effective	September 11, 2001
Patent was granted	September 27, 2005

Biologics License Application (BLA) under PHSA § 351 was submitted	December 6, 2011
BLA was approved	June 8, 2012

(ii) The '245 patent was granted during the period specified in § 156(g)(1)(B)(i) (*i.e.*, the period from the date of the grant of the exemption under § 505(i) of the FDCA until the date of submission of the BLA). Pursuant to § 156(b) and (c)(2), the calculated regulatory review period therefore includes a component of time between when the patent was granted and when the BLA was submitted (1/2 of 2,261 days or 1,131 days).

(iii) The patent was granted prior to the start of the period specified in § 156(g)(1)(B)(ii) (*i.e.*, the period from the date of submission of the BLA until the date of approval). The regulatory review period under § 156(b) therefore includes a component of time between when the BLA was submitted and when the BLA was approved (186 days).

(iv) The period determined according to § 156(b), (c)(2), and (g)(1) for the approved product is 1,317 days.

(v) The '245 patent will expire on May 24, 2021.

(vi) The date of approval of the approved product is June 8, 2012.

(vii) The date that is fourteen years from the date of approval of the approved product is June 8, 2026.

(viii) The date that is provided by adding the number of days determined according to § 156(b), (c)(2), and (g)(1) for the approved product (1,317 days) to the expiration date of the '245 patent is December 31, 2024.

(ix) The date that is fourteen years from the date of approval of the approved product (June 8, 2026) is longer than the date that is provided by adding the number of days determined according to § 156(b), (c)(2), and (g)(1) for the approved product (1,317 days) to the expiration date of the '245 patent (December 31, 2024). As such, the period by which the patent may be extended is not limited by the fourteen-year rule of §156(c)(3).

(x) The '245 patent issued after the effective date of Public Law No. 98-417. As such, the two- or three-year limit of 35 U.S.C. § 156(g)(6)(C) does not apply.

13. Statement Pursuant to 37 C.F.R. § 1.740(a)(13)

Pursuant to 37 C.F.R. § 1.740(a)(13), Applicant acknowledges its duty to disclose to the Director of the PTO and to the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought, particularly as that duty is defined in 37 C.F.R. § 1.765.

14. Applicable Fee [§ 1.740(a)(14)]

Payment of the fee prescribed in 37 C.F.R. § 1.20(j) for a patent term extension application under 35 U.S.C. § 156 is authorized to be charged against deposit account no. 03-1952 referencing docket number 146392015800. The undersigned also authorizes any additional required fees to be deducted from, or any overpayments to be credited to, deposit account no. 03-1952.

15. Name and Address for Correspondence [§ 1.740(a)(15)]

Please direct all inquiries, questions, and communications regarding this application for term extension to:

Catherine M. Polizzi
Registration No.: 40,130
MORRISON & FOERSTER LLP
755 Page Mill Road
Palo Alto, California 94304-1018
650/813-5651

The correspondence address for U.S. Patent No. 6,949,245 is unchanged for all other purposes. A Power of Attorney granted to the Practitioners associated with Customer Number 25226 by the patent Assignee, a copy of which is included with this application as Attachment J, accompanies this communication.

Two additional copies of this application are enclosed, in compliance with 37 C.F.R. § 1.740(b). Applicant also provides herewith two further copies of the application for the convenience of the Office, pursuant to M.P.E.P. § 2763.

Sincerely,

Catherine M. Polizzi
Registration No.: 40,130
MORRISON & FOERSTER LLP
755 Page Mill Road
Palo Alto, California 94304-1018
Phone: 650/813-5651
Facsimile: 650/494-0792

Dated: August 2, 2012

INDEX OF ATTACHMENTS

Attachment A

Pertuzumab Product Label

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use PERJETA safely and effectively. See full prescribing information for PERJETA.

PERJETA™ (pertuzumab)
Injection, for intravenous use
Initial U.S. Approval: 2012

WARNING: EMBRYO-FETAL TOXICITY
See full prescribing information for complete boxed warning.

Exposure to PERJETA can result in embryo-fetal death and birth defects. Studies in animals have resulted in oligohydramnios, delayed renal development, and death. Advise patients of these risks and the need for effective contraception. (5.1, 8.1, 8.6)

INDICATIONS AND USAGE

PERJETA is a HER2/neu receptor antagonist indicated in combination with trastuzumab and docetaxel for the treatment of patients with HER2-positive metastatic breast cancer who have not received prior anti-HER2 therapy or chemotherapy for metastatic disease. (1)

DOSAGE AND ADMINISTRATION

- **For intravenous infusion only.** Do not administer as an intravenous push or bolus. (2.3)
- The initial dose is 840 mg administered as a 60-minute intravenous infusion, followed every 3 weeks thereafter by 420 mg administered as a 30 to 60 minute intravenous infusion. (2.1)

DOSAGE FORMS AND STRENGTHS

- 420 mg/14 mL single-use vial. (3)

CONTRAINDICATIONS

None. (4)

WARNINGS AND PRECAUTIONS

- Embryo-fetal toxicity: Fetal harm can occur when administered to a pregnant woman. (5.1, 8.1)
- Left Ventricular Dysfunction: Monitor LVEF and withhold dosing as appropriate. (5.2, 6.1)
- Infusion-Associated Reactions, Hypersensitivity Reactions/Anaphylaxis: Monitor for signs and symptoms. If a significant infusion-associated reaction occurs, slow or interrupt the infusion and administer appropriate medical therapies. (5.3)
- HER2 testing: Perform using FDA-approved tests by laboratories with demonstrated proficiency. (5.4)

ADVERSE REACTIONS

The most common adverse reactions (> 30%) with PERJETA in combination with trastuzumab and docetaxel were diarrhea, alopecia, neutropenia, nausea, fatigue, rash, and peripheral neuropathy. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Genentech at 1-888-835-2555 or FDA at 1-800-FDA-1088 or *www.fda.gov/medwatch*.

USE IN SPECIFIC POPULATIONS

- Nursing mothers: Discontinue nursing or discontinue PERJETA, taking into consideration the importance of the drug to the mother. (8.3)
- Females of Reproductive Potential: Counsel females on pregnancy prevention and planning. Encourage patient participation in the MotHER Pregnancy Registry by contacting 1-800-690-6720. (5.1, 8.1, 8.6, 17)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 06/2012

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the Full Prescribing Information are not listed.

FULL PRESCRIBING INFORMATION

WARNING: EMBRYO-FETAL TOXICITY

Exposure to PERJETA can result in embryo-fetal death and birth defects. Studies in animals have resulted in oligohydramnios, delayed renal development, and death. Advise patients of these risks and the need for effective contraception. (5.1, 8.1, 8.6)

1 INDICATIONS AND USAGE

PERJETA is indicated for use in combination with trastuzumab and docetaxel for the treatment of patients with HER2-positive metastatic breast cancer who have not received prior anti-HER2 therapy or chemotherapy for metastatic disease.

2 DOSAGE AND ADMINISTRATION

2.1 Recommended Doses and Schedules

The initial dose of PERJETA is 840 mg administered as a 60-minute intravenous infusion, followed every 3 weeks thereafter by a dose of 420 mg administered as an intravenous infusion over 30 to 60 minutes.

When administered with PERJETA, the recommended initial dose of trastuzumab is 8 mg/kg administered as a 90-minute intravenous infusion, followed every 3 weeks thereafter by a dose of 6 mg/kg administered as an intravenous infusion over 30 to 90 minutes.

When administered with PERJETA, the recommended initial dose of docetaxel is 75 mg/m^2 administered as an intravenous infusion. The dose may be escalated to 100 mg/m^2 administered every 3 weeks if the initial dose is well tolerated.

2.2 Dose Modification

For delayed or missed doses, if the time between two sequential infusions is less than 6 weeks, the 420 mg dose of PERJETA should be administered. Do not wait until the next planned dose. If the time between two sequential infusions is 6 weeks or more, the initial dose of 840 mg PERJETA should be re-administered as a 60-minute intravenous infusion followed every 3 weeks thereafter by a dose of 420 mg administered as an intravenous infusion over 30 to 60 minutes.

The infusion rate of PERJETA may be slowed or interrupted if the patient develops an infusion-associated reaction. The infusion should be discontinued immediately if the patient experiences a serious hypersensitivity reaction *[see Warnings and Precautions (5.2)]*.

Left Ventricular Ejection Fraction (LVEF):

Withhold PERJETA and trastuzumab dosing for at least 3 weeks for either:

- a drop in LVEF to less than 40% or
- LVEF of 40% to 45% with a 10% or greater absolute decrease below pretreatment values *[see Warnings and Precautions (5.2)]*

PERJETA may be resumed if the LVEF has recovered to greater than 45% or to 40% to 45% associated with less than a 10% absolute decrease below pretreatment values.

If after a repeat assessment within approximately 3 weeks, the LVEF has not improved, or has declined further, discontinuation of PERJETA and trastuzumab should be strongly considered,

unless the benefits for the individual patient are deemed to outweigh the risks *[see Warnings and Precautions (5.2)]*.

PERJETA should be withheld or discontinued if trastuzumab treatment is withheld or discontinued.

If docetaxel is discontinued, treatment with PERJETA and trastuzumab may continue.

Dose reductions are not recommended for PERJETA.

For docetaxel dose modifications, see docetaxel prescribing information.

2.3 Preparation for Administration

Administer as an intravenous infusion only. Do not administer as an intravenous push or bolus. Do not mix PERJETA with other drugs.

Preparation

Prepare the solution for infusion, using aseptic technique, as follows:

- Parenteral drug products should be inspected visually for particulates and discoloration prior to administration.
- Withdraw the appropriate volume of PERJETA solution from the vial(s).
- Dilute into a 250 mL 0.9% sodium chloride PVC or non-PVC polyolefin infusion bag.
- Mix diluted solution by gentle inversion. Do not shake.
- Administer immediately once prepared.
- If the diluted infusion solution is not used immediately, it can be stored at 2°C to 8°C for up to 24 hours.
- Dilute with 0.9% Sodium Chloride injection only. Do not use dextrose (5%) solution.

3 DOSAGE FORMS AND STRENGTHS

PERJETA (pertuzumab) 420 mg/14 mL (30 mg/mL) in a single-use vial

4 CONTRAINDICATIONS

None.

5 WARNINGS AND PRECAUTIONS

5.1 Embryo-Fetal Toxicity

PERJETA can cause fetal harm when administered to a pregnant woman. Treatment of pregnant cynomolgus monkeys with pertuzumab resulted in oligohydramnios, delayed fetal kidney development, and embryo-fetal death. If PERJETA is administered during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to a fetus *[see Use in Specific Populations (8.1)]*.

Verify pregnancy status prior to the initiation of PERJETA. Advise patients of the risks of embryo-fetal death and birth defects and the need for contraception during and after treatment. Advise patients to contact their healthcare provider immediately if they suspect they may be pregnant. If PERJETA is administered during pregnancy or if a patient becomes pregnant while receiving PERJETA, immediately report exposure to the Genentech Adverse Event Line at 1-888-835-2555. Encourage women who may be exposed during pregnancy to enroll in the MotHER Pregnancy Registry by contacting 1-800-690-6720 *[see Patient Counseling Information (17)]*.

Monitor patients who become pregnant during PERJETA therapy for oligohydramnios. If oligohydramnios occurs, perform fetal testing that is appropriate for gestational age and consistent with community standards of care. The efficacy of intravenous hydration in the management of oligohydramnios due to PERJETA exposure is not known.

5.2 Left Ventricular Dysfunction

Decreases in LVEF have been reported with drugs that block HER2 activity, including PERJETA. In the randomized trial, PERJETA in combination with trastuzumab and docetaxel was not associated with increases in the incidence of symptomatic left ventricular systolic dysfunction (LVSD) or decreases in LVEF compared with placebo in combination with trastuzumab and docetaxel *[see Clinical Studies (14.1)]*. Left ventricular dysfunction occurred in 4.4% of patients in the PERJETA-treated group and 8.3% of patients in the placebo-treated group. Symptomatic left ventricular systolic dysfunction (congestive heart failure) occurred in 1.0% of patients in the PERJETA-treated group and 1.8% of patients in the placebo-treated group *[see Adverse Reactions (6.1)]*. Patients who have received prior anthracyclines or prior radiotherapy to the chest area may be at higher risk of decreased LVEF.

PERJETA has not been studied in patients with a pretreatment LVEF value of ≤ 50%, a prior history of CHF, decreases in LVEF to < 50% during prior trastuzumab therapy, or conditions that could impair left ventricular function such as uncontrolled hypertension, recent myocardial infarction, serious cardiac arrhythmia requiring treatment or a cumulative prior anthracycline exposure to > 360 mg/m^2 of doxorubicin or its equivalent.

Assess LVEF prior to initiation of PERJETA and at regular intervals (e.g., every three months) during treatment to ensure that LVEF is within the institution's normal limits. If LVEF is < 40%, or is 40% to 45% with a 10% or greater absolute decrease below the pretreatment value, withhold PERJETA and trastuzumab and repeat LVEF assessment within approximately 3 weeks. Discontinue PERJETA and trastuzumab if the LVEF has not improved or has declined further, unless the benefits for the individual patient outweigh the risks *[see Dosage and Administration (2.2)]*.

5.3 Infusion-Associated Reactions, Hypersensitivity Reactions/Anaphylaxis

PERJETA has been associated with infusion and hypersensitivity reactions *[see Adverse Reactions (6.1)]*. An infusion reaction was defined in the randomized trial as any event described as hypersensitivity, anaphylactic reaction, acute infusion reaction or cytokine release syndrome occurring during an infusion or on the same day as the infusion. The initial dose of PERJETA was given the day before trastuzumab and docetaxel to allow for the examination of PERJETA-associated reactions. On the first day, when only PERJETA was administered, the overall frequency of infusion reactions was 13.0% in the PERJETA-treated group and 9.8% in the placebo-treated group. Less than 1% were grade 3 or 4. The most common infusion reactions (≥ 1.0%) were pyrexia, chills, fatigue, headache, asthenia, hypersensitivity, and vomiting.

During the second cycle when all drugs were administered on the same day, the most common infusion reactions in the PERJETA-treated group (≥ 1.0%) were fatigue, dysgeusia, hypersensitivity, myalgia, and vomiting.

In the randomized trial, the overall frequency of hypersensitivity/anaphylaxis reactions was 10.8% in the PERJETA-treated group and 9.1% in the placebo-treated group. The incidence of Grade 3 – 4 hypersensitivity/anaphylaxis reactions was 2% in the PERJETA-treated group and 2.5% in the placebo-treated group according to National Cancer Institute – Common

Terminology Criteria for Adverse Events (NCI - CTCAE) (version 3). Overall, 4 patients in PERJETA-treated group and 2 patients in the placebo-treated group experienced anaphylaxis.

Observe patients closely for 60 minutes after the first infusion and for 30 minutes after subsequent infusions of PERJETA. If a significant infusion-associated reaction occurs, slow or interrupt the infusion and administer appropriate medical therapies. Monitor patients carefully until complete resolution of signs and symptoms. Consider permanent discontinuation in patients with severe infusion reactions *[see Dosage and Administration (2.2)]*.

5.4 HER2 Testing

Detection of HER2 protein overexpression is necessary for selection of patients appropriate for PERJETA therapy because these are the only patients studied and for whom benefit has been shown *[see Indications and Usage (1) and Clinical Studies (14)]*. In the randomized trial, patients with breast cancer were required to have evidence of HER2 overexpression defined as 3+ IHC by Dako Herceptest™ or FISH amplification ratio ≥ 2.0 by Dako *HER2* FISH PharmDx™ test kit. Only limited data were available for patients whose breast cancer was positive by FISH, but did not demonstrate protein overexpression by IHC.

Assessment of HER2 status should be performed by laboratories with demonstrated proficiency in the specific technology being utilized. Improper assay performance, including use of sub-optimally fixed tissue, failure to utilize specified reagents, deviation from specific assay instructions, and failure to include appropriate controls for assay validation, can lead to unreliable results.

6 ADVERSE REACTIONS

The following adverse reactions are discussed in greater detail in other sections of the label:

- Embryo-Fetal Toxicity *[see Warnings and Precautions (5.1)]*
- Left Ventricular Dysfunction *[see Warnings and Precautions (5.2)]*
- Infusion-Associated Reactions, Hypersensitivity Reactions/Anaphylaxis *[see Warnings and Precautions (5.3)]*

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in clinical practice.

In clinical trials, PERJETA has been evaluated in more than 1400 patients with various malignancies and treatment with PERJETA was predominantly in combination with other anti-neoplastic agents.

The adverse reactions described in Table 1 were identified in 804 patients with HER2-positive metastatic breast cancer treated in the randomized trial. Patients were randomized to receive either PERJETA in combination with trastuzumab and docetaxel or placebo in combination with trastuzumab and docetaxel. The median duration of study treatment was 18.1 months for patients in the PERJETA-treated group and 11.8 months for patients in the placebo-treated group. No dose adjustment was permitted for PERJETA or trastuzumab. The rates of adverse events resulting in permanent discontinuation of all study therapy were 6.1% for patients in the PERJETA-treated group and 5.3% for patients in the placebo-treated group. Adverse events led to discontinuation of docetaxel alone in 23.6% of patients in the PERJETA-treated group and 23.2% of patients in the placebo-treated group. Table 1 reports the adverse reactions that occurred in at least 10% of patients in the PERJETA-treated group.

The most common adverse reactions (> 30%) seen with PERJETA in combination with trastuzumab and docetaxel were diarrhea, alopecia, neutropenia, nausea, fatigue, rash, and peripheral neuropathy. The most common NCI - CTCAE (version 3) Grade 3 – 4 adverse reactions (> 2%) were neutropenia, febrile neutropenia, leukopenia, diarrhea, peripheral neuropathy, anemia, asthenia, and fatigue. An increased incidence of febrile neutropenia was observed for Asian patients in both treatment arms compared with patients of other races and from other geographic regions. Among Asian patients, the incidence of febrile neutropenia was higher in the pertuzumab-treated group (26%) compared with the placebo-treated group (12%).

Table 1 Summary of Adverse Reactions Occurring in ≥ 10% of Patients on the PERJETA Treatment Arm in the Randomized Trial

Body System/Adverse Reactions	PERJETA + trastuzumab + docetaxel n=407 Frequency rate %		Placebo + trastuzumab + docetaxel n=397 Frequency rate %	
	All Grades %	Grades 3 – 4 %	All Grades %	Grades 3 – 4 %
General disorders and administration site conditions				
Fatigue	37.6	2.2	36.8	3.3
Asthenia	26.0	2.5	30.2	1.5
Edema peripheral	23.1	0.5	30.0	0.8
Mucosal inflammation	27.8	1.5	19.9	1.0
Pyrexia	18.7	1.2	17.9	0.5
Skin and subcutaneous tissue disorders				
Alopecia	60.9	0.0	60.5	0.3
Rash	33.7	0.7	24.2	0.8
Nail disorder	22.9	1.2	22.9	0.3
Pruritus	14.0	0.0	10.1	0.0
Dry skin	10.6	0.0	4.3	0.0
Gastrointestinal disorders				
Diarrhea	66.8	7.9	46.3	5.0
Nausea	42.3	1.2	41.6	0.5
Vomiting	24.1	1.5	23.9	1.5
Constipation	15.0	0.0	24.9	1.0
Stomatitis	18.9	0.5	15.4	0.3
Blood and lymphatic system disorders				
Neutropenia	52.8	48.9	49.6	45.8
Anemia	23.1	2.5	18.9	3.5
Leukopenia	18.2	12.3	20.4	14.6
Febrile neutropenia*	13.8	13.0	7.6	7.3

Nervous system disorders				
Neuropathy peripheral	32.4	3.2	33.8	2.0
Headache	20.9	1.2	16.9	0.5
Dysgeusia	18.4	0.0	15.6	0.0
Dizziness	12.5	0.5	12.1	0.0
Musculoskeletal and connective tissue disorders				
Myalgia	22.9	1.0	23.9	0.8
Arthralgia	15.5	0.2	16.1	0.8
Infections and infestations				
Upper respiratory tract infection	16.7	0.7	13.4	0.0
Nasopharyngitis	11.8	0.0	12.8	0.3
Respiratory, thoracic and mediastinal disorders				
Dyspnea	14.0	1.0	15.6	2.0
Metabolism and nutrition disorders				
Decreased appetite	29.2	1.7	26.4	1.5
Eye disorders				
Lacrimation increased	14.0	0.0	13.9	0.0
Psychiatric disorders				
Insomnia	13.3	0.0	13.4	0.0

* In this table this denotes an adverse reaction that has been reported in association with a fatal outcome

The following clinically relevant adverse reactions were reported in < 10% of patients in the PERJETA-treated group:

Skin and subcutaneous tissue disorders: Paronychia (7.1% in the PERJETA-treated group vs. 3.5% in the placebo-treated group)

Respiratory, thoracic and mediastinal disorders: Pleural effusion (5.2% in the PERJETA-treated group vs. 5.8% in the placebo-treated group)

Cardiac disorders: Left ventricular dysfunction (4.4% in the PERJETA-treated group vs. 8.3% in the placebo-treated group) including symptomatic left ventricular systolic dysfunction (CHF) (1.0% in the PERJETA-treated group vs. 1.8% in the placebo-treated group)

Immune system disorders: Hypersensitivity (10.1% in the PERJETA-treated group vs. 8.6% in placebo-treated group)

Adverse Reactions Reported in Patients Receiving PERJETA and Trastuzumab after Discontinuation of Docetaxel

In the randomized trial, adverse reactions were reported less frequently after discontinuation of docetaxel treatment. All adverse reactions in the PERJETA and trastuzumab treatment group

occurred in < 10% of patients with the exception of diarrhea (19.1%), upper respiratory tract infection (12.8%), rash (11.7%), headache (11.4%), and fatigue (11.1%).

6.2 Immunogenicity

As with all therapeutic proteins, there is the potential for an immune response to PERJETA.

Patients in the randomized trial were tested at multiple time-points for antibodies to PERJETA. Approximately 2.8% (11/386) of patients in the PERJETA-treated group and 6.2% (23/372) of patients in the placebo-treated group tested positive for anti-PERJETA antibodies. Of these 34 patients, none experienced anaphylactic/hypersensitivity reactions that were clearly related to the anti-therapeutic antibodies (ATA). The presence of pertuzumab in patient serum at the levels expected at the time of ATA sampling can interfere with the ability of this assay to detect anti-pertuzumab antibodies. In addition, the assay may be detecting antibodies to trastuzumab. As a result, data may not accurately reflect the true incidence of anti-pertuzumab antibody development.

Immunogenicity data are highly dependent on the sensitivity and specificity of the test methods used. Additionally, the observed incidence of a positive result in a test method may be influenced by several factors, including sample handling, timing of sample collection, drug interference, concomitant medication, and the underlying disease. For these reasons, comparison of the incidence of antibodies to PERJETA with the incidence of antibodies to other products may be misleading.

7 DRUG INTERACTIONS

No drug-drug interactions were observed between pertuzumab and trastuzumab, or between pertuzumab and docetaxel.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category D

Risk Summary

There are no adequate and well-controlled studies of PERJETA in pregnant women. Based on findings in animal studies, PERJETA can cause fetal harm when administered to a pregnant woman. The effects of PERJETA are likely to be present during all trimesters of pregnancy. Pertuzumab administered to pregnant cynomolgus monkeys resulted in oligohydramnios, delayed fetal kidney development, and embryo-fetal deaths at clinically relevant exposures of 2.5 to 20-fold greater than the recommended human dose, based on C_{max}. If PERJETA is administered during pregnancy, or if a patient becomes pregnant while receiving PERJETA, the patient should be apprised of the potential hazard to the fetus.

If PERJETA is administered during pregnancy or if a patient becomes pregnant while receiving PERJETA, immediately report exposure to the Genentech Adverse Event Line at 1-888-835-2555. Encourage women who may be exposed during pregnancy to enroll in the MotHER Pregnancy Registry by contacting 1-800-690-6720 *[see Patient Counseling Information (17)]*.

Animal Data

Reproductive toxicology studies have been conducted in cynomolgus monkeys. Pregnant monkeys were treated on Gestational Day (GD)19 with loading doses of 30 to 150 mg/kg pertuzumab, followed by bi-weekly doses of 10 to 100 mg/kg. These dose levels resulted in clinically relevant exposures of 2.5 to 20-fold greater than the recommended human dose, based

on C_{max}. Intravenous administration of pertuzumab from GD19 through GD50 (period of organogenesis) was embryotoxic, with dose-dependent increases in embryo-fetal death between GD25 to GD70. The incidences of embryo-fetal loss were 33, 50, and 85% for dams treated with bi-weekly pertuzumab doses of 10, 30, and 100 mg/kg, respectively (2.5 to 20-fold greater than the recommended human dose, based on C_{max}). At Caesarean section on GD100, oligohydramnios, decreased relative lung and kidney weights and microscopic evidence of renal hypoplasia consistent with delayed renal development were identified in all pertuzumab dose groups. Pertuzumab exposure was reported in offspring from all treated groups, at levels of 29% to 40% of maternal serum levels at GD100.

8.3 Nursing Mothers

It is not known whether PERJETA is excreted in human milk, but human IgG is excreted in human milk. Because many drugs are secreted in human milk and because of the potential for serious adverse reactions in nursing infants from PERJETA, a decision should be made whether to discontinue nursing, or discontinue drug, taking into account the elimination half-life of PERJETA and the importance of the drug to the mother *[See Warnings and Precautions (5.1), Clinical Pharmacology (12.3)]*.

8.4 Pediatric Use

The safety and effectiveness of PERJETA have not been established in pediatric patients.

8.5 Geriatric Use

Of 402 patients who received PERJETA in the randomized trial, 60 patients (15%) were ≥ 65 years of age and 5 patients (1%) were ≥ 75 years of age. No overall differences in efficacy and safety of PERJETA were observed between these patients and younger patients.

Based on a population pharmacokinetic analysis, no significant difference was observed in the pharmacokinetics of pertuzumab between patients < 65 years (n=306) and patients ≥ 65 years (n=175).

8.6 Females of Reproductive Potential

PERJETA can cause embryo-fetal harm when administered during pregnancy. Counsel patients regarding pregnancy prevention and planning. Advise females of reproductive potential to use effective contraception while receiving PERJETA and for 6 months following the last dose of PERJETA.

If PERJETA is administered during pregnancy or if a patient becomes pregnant while receiving PERJETA, immediately report exposure to the Genentech Adverse Event Line at 1-888-835-2555. Encourage women who may be exposed during pregnancy to enroll in the MotHER Pregnancy Registry by contacting1-800-690-6720 *[see Patient Counseling Information (17)]*.

8.7 Renal Impairment

Dose adjustments of PERJETA are not needed in patients with mild (creatinine clearance [CLcr] 60 to 90 mL/min) or moderate (CLcr 30 to 60 mL/min) renal impairment. No dose adjustment can be recommended for patients with severe renal impairment (CLcr less than 30 mL/min) because of the limited pharmacokinetic data available *[see Clinical Pharmacology (12.3)]*.

8.8 Hepatic Impairment

No clinical studies have been conducted to evaluate the effect of hepatic impairment on the pharmacokinetics of pertuzumab.

10 OVERDOSAGE

No drug overdoses have been reported with PERJETA to date.

11 DESCRIPTION

Pertuzumab is a recombinant humanized monoclonal antibody that targets the extracellular dimerization domain (Subdomain II) of the human epidermal growth factor receptor 2 protein (HER2). Pertuzumab is produced by recombinant DNA technology in a mammalian cell (Chinese Hamster Ovary) culture containing the antibiotic, gentamicin. Gentamicin is not detectable in the final product. Pertuzumab has an approximate molecular weight of 148 kDa.

PERJETA is a sterile, clear to slightly opalescent, colorless to pale brown liquid for intravenous infusion. Each single use vial contains 420 mg of pertuzumab at a concentration of 30 mg/mL in 20 mM L-histidine acetate (pH 6.0), 120 mM sucrose and 0.02% polysorbate 20.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Pertuzumab targets the extracellular dimerization domain (Subdomain II) of the human epidermal growth factor receptor 2 protein (HER2) and, thereby, blocks ligand-dependent heterodimerization of HER2 with other HER family members, including EGFR, HER3 and HER4. As a result, pertuzumab inhibits ligand-initiated intracellular signaling through two major signal pathways, mitogen-activated protein (MAP) kinase and phosphoinositide 3-kinase (PI3K). Inhibition of these signaling pathways can result in cell growth arrest and apoptosis, respectively. In addition, pertuzumab mediates antibody-dependent cell-mediated cytotoxicity (ADCC).

While pertuzumab alone inhibited the proliferation of human tumor cells, the combination of pertuzumab and trastuzumab significantly augmented anti-tumor activity in HER2-overexpressing xenograft models.

12.3 Pharmacokinetics

Pertuzumab demonstrated linear pharmacokinetics at a dose range of 2 – 25 mg/kg. Based on a population PK analysis that included 481 patients, the median clearance (CL) of pertuzumab was 0.24 L/day and the median half-life was 18 days. With an initial dose of 840 mg followed by a maintenance dose of 420 mg every three weeks thereafter, the steady-state concentration of pertuzumab was reached after the first maintenance dose.

The population PK analysis suggested no PK differences based on age, gender, and ethnicity (Japanese vs. non-Japanese). Baseline serum albumin level and lean body weight as covariates only exerted a minor influence on PK parameters. Therefore, no dose adjustments based on body weight or baseline albumin level are needed.

No drug-drug interactions were observed between pertuzumab and trastuzumab, or between pertuzumab and docetaxel in a sub-study of 37 patients in the randomized trial.

No dedicated renal impairment trial for PERJETA has been conducted. Based on the results of the population pharmacokinetic analysis, pertuzumab exposure in patients with mild (CLcr 60 to 90 mL/min, n=200) and moderate renal impairment (CLcr 30 to 60 mL/min, n=71) were similar to those in patients with normal renal function (CLcr greater than 90 mL/min, n=200). No relationship between CLcr and pertuzumab exposure was observed over the range of observed CLcr (27 to 244 mL/min).

12.6 Cardiac Electrophysiology

The effect of pertuzumab with an initial dose of 840 mg followed by a maintenance dose of

420 mg every three weeks on QTc interval was evaluated in a subgroup of 20 patients with HER2-positive breast cancer in the randomized trial. No large changes in the mean QT interval (i.e., greater than 20 ms) from placebo based on Fridericia correction method were detected in the trial. A small increase in the mean QTc interval (i.e., less than 10 ms) cannot be excluded because of the limitations of the trial design.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals have not been performed to evaluate the carcinogenic potential of pertuzumab.

Studies have not been performed to evaluate the mutagenic potential of pertuzumab.

No specific fertility studies in animals have been performed to evaluate the effect of pertuzumab. No adverse effects on male and female reproductive organs were observed in repeat-dose toxicity studies of up to six months duration in cynomolgus monkeys.

14 CLINICAL STUDIES

14.1 Metastatic Breast Cancer

The randomized trial was a multicenter, double-blind, placebo-controlled trial of 808 patients with HER2-positive metastatic breast cancer. Breast tumor specimens were required to show HER2 overexpression defined as 3+ IHC or FISH amplification ratio ≥ 2.0 determined at a central laboratory. Patients were randomized 1:1 to receive placebo plus trastuzumab and docetaxel or PERJETA plus trastuzumab and docetaxel. Randomization was stratified by prior treatment (prior or no prior adjuvant/neoadjuvant anti-HER2 therapy or chemotherapy) and geographic region (Europe, North America, South America, and Asia). Patients with prior adjuvant or neoadjuvant therapy were required to have a disease-free interval of greater than 12 months before trial enrollment.

PERJETA was given intravenously at an initial dose of 840 mg, followed by 420 mg every 3 weeks thereafter. Trastuzumab was given intravenously at an initial dose of 8 mg/kg, followed by 6 mg/kg every 3 weeks thereafter. Patients were treated with PERJETA and trastuzumab until progression of disease, withdrawal of consent, or unacceptable toxicity. Docetaxel was given as an initial dose of 75 mg/m^2 by intravenous infusion every 3 weeks for at least 6 cycles. The docetaxel dose could be escalated to 100 mg/m^2 at the investigator's discretion if the initial dose was well tolerated. At the time of the primary analysis, the mean number of cycles of study treatment administered was 16.2 in the placebo-treated group and 19.9 in the PERJETA-treated group.

The primary endpoint of the randomized trial was progression-free survival (PFS) as assessed by an independent review facility (IRF). PFS was defined as the time from the date of randomization to the date of disease progression or death (from any cause) if the death occurred within 18 weeks of the last tumor assessment. Additional endpoints included overall survival (OS), PFS (investigator-assessed), objective response rate (ORR) and duration of response.

Patient demographic and baseline characteristics were balanced between the treatment arms. The median age was 54 (range 22 to 89 years), 59% were White, 32% were Asian, and 4% were Black. All were women with the exception of 2 patients. Seventeen percent of patients were enrolled in North America, 14% in South America, 38% in Europe, and 31% in Asia. Tumor prognostic characteristics, including hormone receptor status (positive 48%, negative 50%), presence of visceral disease (78%) and non-visceral disease only (22%) were similar in the study arms. Approximately half of the patients received prior adjuvant or neoadjuvant anti-HER2

therapy or chemotherapy (placebo 47%, PERJETA 46%). Among patients with hormone receptor positive tumors, 45% received prior adjuvant hormonal therapy and 11% received hormonal therapy for metastatic disease. Eleven percent of patients received prior adjuvant or neoadjuvant trastuzumab.

The randomized trial demonstrated a statistically significant improvement in IRF-assessed PFS in the PERJETA-treated group compared with the placebo-treated group [hazard ratio (HR) = 0.62 (95% CI: 0.51, 0.75), $p < 0.0001$] and an increase in median PFS of 6.1 months (median PFS of 18.5 months in the PERJETA-treated group vs. 12.4 months in the placebo-treated group) (see Figure 1). The results for investigator-assessed PFS were comparable to those observed for IRF-assessed PFS.

Consistent results were observed across several patient subgroups including age (< 65 or ≥ 65 years), race, geographic region, prior adjuvant/neoadjuvant anti-HER2 therapy or chemotherapy (yes or no), and prior adjuvant/neoadjuvant trastuzumab (yes or no). In the subgroup of patients with hormone receptor-negative disease (n=408), the hazard ratio was 0.55 (95% CI: 0.42, 0.72). In the subgroup of patients with hormone receptor-positive disease (n=388), the hazard ratio was 0.72 (95% CI: 0.55, 0.95). In the subgroup of patients with disease limited to non-visceral metastasis (n=178), the hazard ratio was 0.96 (95% CI: 0.61, 1.52).

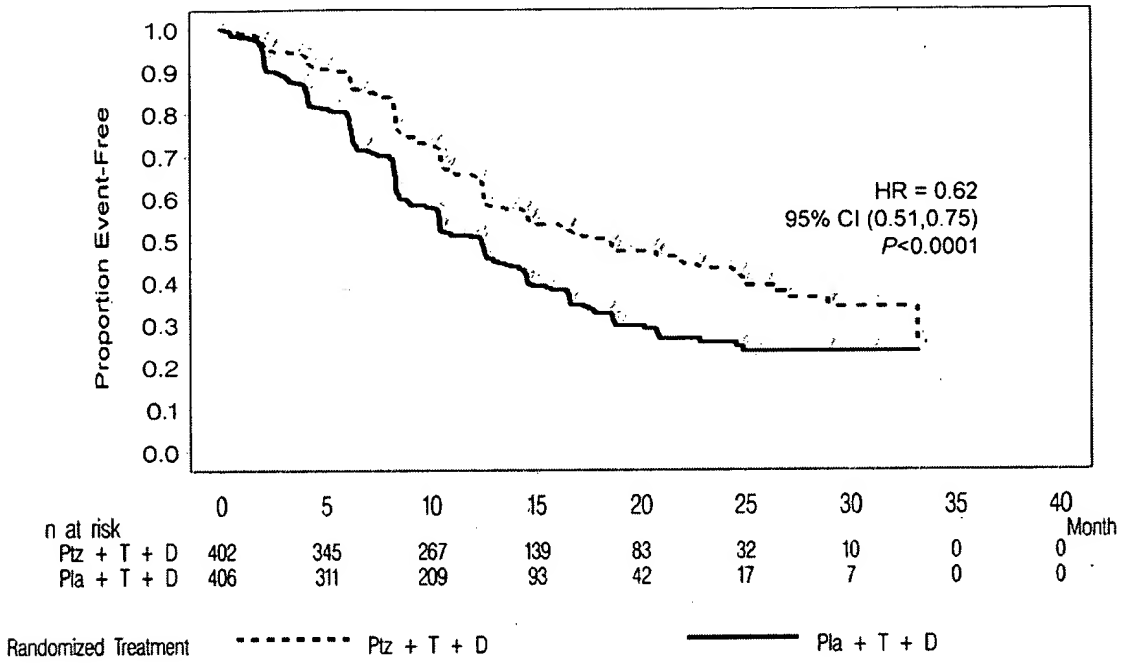
At the time of the PFS analysis, 165 patients had died. More deaths occurred in the placebo-treated group (23.6%) compared with the PERJETA-treated group (17.2%). At the interim OS analysis, the results were not mature and did not meet the pre-specified stopping boundary for statistical significance. See Table 2 and Figure 2.

Table 2 Summary of Efficacy from the Randomized Trial

Parameter	PERJETA + trastuzumab + docetaxel n=402	Placebo + trastuzumab + docetaxel n=406	HR (95% CI)	p-value
Progression-Free Survival (independent review)			0.62 (0.51, 0.75)	< 0.0001
No. of patients with an event	191 (47.5%)	242 (59.6%)		
Median months	18.5	12.4		
Overall Survival (interim analysis)			0.64 (0.47, 0.88)	0.0053*
No. of patients with an event	69 (17.2%)	96 (23.6%)		
Objective Response Rate (ORR)				
No. of patients analyzed	343	336		
Objective response (CR + PR)	275 (80.2%)	233 (69.3%)		
Complete response (CR)	19 (5.5%)	14 (4.2%)		
Partial Response (PR)	256 (74.6%)	219 (65.2%)		
Median Duration of Response (months)	20.2	12.5		

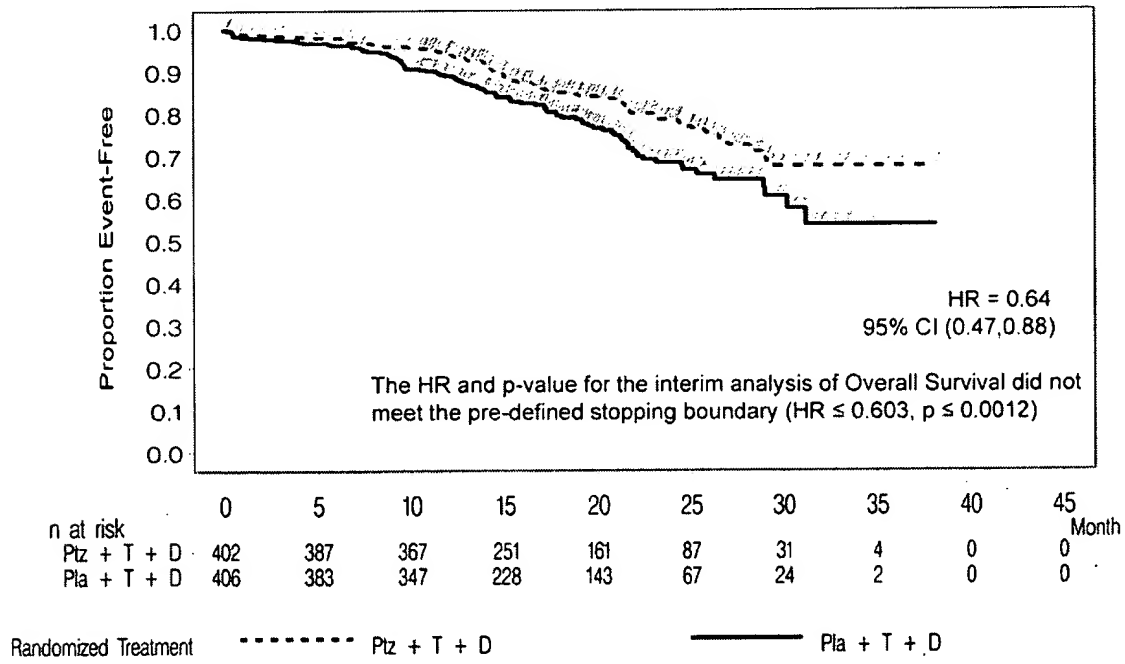
* The HR and p-value for the interim analysis of Overall Survival did not meet the pre-defined stopping boundary (HR ≤ 0.603, p ≤ 0.0012).

Figure 1 **Kaplan-Meier Curve of IRF-Assessed Progression-Free Survival for the Randomized Trial**



Ptz + T + D = Pertuzumab + Trastuzumab + Docetaxel
Pla + T + D = Placebo + Trastuzumab + Docetaxel

Figure 2 **Kaplan-Meier Curve of Overall Survival for the Randomized Trial**



Ptz + T + D = Pertuzumab + Trastuzumab + Docetaxel
Pla + T + D = Placebo + Trastuzumab + Docetaxel

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

PERJETA is supplied as a 420 mg/14 mL (30 mg/mL) single-use vial containing preservative-free solution. NDC 50242-145-01.

Store vials in a refrigerator at 2°C to 8°C (36°F to 46°F) until time of use.

Keep vial in the outer carton in order to protect from light.

DO NOT FREEZE. DO NOT SHAKE.

17 PATIENT COUNSELING INFORMATION

- Advise pregnant women and females of reproductive potential that PERJETA exposure can result in fetal harm, including embryo-fetal death or birth defects *[see Warnings and Precautions (5.1) and Use in Specific Populations (8.1)]*
- Advise females of reproductive potential to use effective contraception while receiving PERJETA and for 6 months following the last dose of PERJETA *[see Warnings and Precautions (5.1) and Use in Special Populations (8.6)]*
- Advise nursing mothers treated with PERJETA to discontinue nursing or discontinue PERJETA, taking into account the importance of the drug to the mother *[see Use in Specific Populations (8.3)]*.
- Encourage women who are exposed to PERJETA during pregnancy to enroll in the MotHER Pregnancy Registry by contacting 1-800-690-6720 *[see Warnings and Precautions (5.1) and Use in Specific Populations (8.1)]*

PERJETA™ (pertuzumab)

L01XC13

Manufactured by:

Genentech, Inc.

A Member of the Roche Group

1 DNA Way

South San Francisco, CA 94080-4990

Attachment B

Pertuzumab Biologics' License Application (BLA) Approval

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

BLA 125409/0

BLA APPROVAL

Genentech, Inc.
Attention: Josephine Ing
Sr. Scientist, Regulatory Affairs
1 DNA Way
South San Francisco, CA 94080-4990

Dear Ms. Ing:

Please refer to your Biologics License Application (BLA) dated December 6, 2011, received December 8, 2011, submitted under section 351 of the Public Health Service Act for Perjeta (pertuzumab).

We acknowledge receipt of your amendments dated December 6, 2011; January 6, 18, and 23; February 1, 3, 10, 27, and 28; March 1, 8, 9, 12, 15, 16, 21 (3), 22 , and 28; April 2, 3, 9 (2), 10, 12, 16, 19, 23, 26, and 30; May 1, 2, 4 (2), 8, 10 (3), 11 (3), 14 (3), 16 (3), 17 (3), 18, 21 (2), 22, 23, 25, 29, and 31; June 4, 5, and 8 (e-mail), 2012.

We have approved your BLA for pertuzumab effective this date, pursuant to the conditions set forth in this letter. You are hereby authorized to introduce, or deliver for introduction into interstate commerce, pertuzumab under your existing Department of Health and Human Services U.S. License No. 1048, as further described below. Pertuzumab is indicated for use in combination with trastuzumab and docetaxel for the treatment of patients with HER2-positive metastatic breast cancer who have not received prior anti-HER2 therapy or chemotherapy for metastatic disease.

Under this license, you are approved to introduce, or deliver for introduction into interstate commerce, only pertuzumab drug product formulated from drug substance manufactured at Genentech's facility in Vacaville, California, during your 2010 campaign that was not produced from any engineering or failed runs. The final formulated product will be manufactured, filled, labeled, and packaged at Roche Diagnostics GmbH, Mannheim, Germany. You may label your product with the proprietary name Perjeta and will market it in 420 mg per 14 mL single-use vials. You are not licensed to introduce, or deliver for introduction into interstate commerce, any other pertuzumab drug product without the submission and Agency approval of a supplemental BLA for such marketing.

Your application for pertuzumab was not referred to an FDA advisory committee because the Agency did not believe that outside expertise was necessary; there were no controversial clinical issues that would benefit from advisory committee discussion.

The dating period for pertuzumab drug product shall be 24 months from the date of manufacture when stored at 2-8°C. The date of manufacture shall be defined as the date of final sterile filtration of the formulated drug product. The dating period for pertuzumab drug substance shall be 24 months from the date of manufacture when stored at -20°C. We have approved the stability protocols in your license application for the purpose of extending the expiration dating period of your drug substance and drug product under 21 CFR 601.12.

You are not currently required to submit samples of future lots of pertuzumab and each kit component to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor your compliance with 21 CFR 610.1, which provides that you shall not release any lot of licensed product prior to completion of tests for conformity with applicable standards.

Any changes in the manufacturing, testing, packaging, or labeling of pertuzumab, or in the manufacturing facilities, will require the submission of information to your biologics license application for our review and written approval, consistent with 21 CFR 601.12.

We are approving this application for use as recommended in the enclosed agreed-upon labeling text.

POSTMARKETING REQUIREMENTS AND COMMITMENTS

Below we have set forth a summary of the postmarketing requirements and commitments associated with this approval. Further details of these requirements and commitments will be communicated in a separate letter; Genentech has agreed to the postmarking commitments as described more fully in this separate letter.

POSTMARKETING REQUIREMENTS UNDER 505(o)

Section 505(o)(3) of the Federal Food, Drug, and Cosmetic Act (FDCA) authorizes FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute.

We have determined that an analysis of spontaneous postmarketing adverse events reported under subsection 505(k)(1) of the FDCA will not be sufficient to assess a signal of the serious risk of embryo-fetal toxicity.

Furthermore, the new pharmacovigilance system that FDA is required to establish under section 505(k)(3) of the FDCA will not be sufficient to assess this serious risk.

Therefore, based on appropriate scientific data, FDA has determined that you are required to:

1. Establish a Pregnancy Registry to collect and analyze information for ten years on pregnancy complications and birth outcomes in women with breast cancer exposed to a pertuzumab-containing regimen within 6 months of conception or during pregnancy. Submit yearly

interim reports, which may be included in your annual reports, on the cumulative findings and analyses from the Pregnancy Registry.

The timetable you submitted on May 16, 2012, states that you will conduct this study according to the following schedule:

Draft Protocol Submission:	06/2012
Final Protocol Submission:	08/2012
Interim Report #1:	08/2013
Interim Report #2:	08/2014
Interim Report #3:	08/2015
Interim Report #4	08/2016
Interim Report #5	08/2017
Interim Report #6	08/2018
Interim Report #7	08/2019
Interim Report #8	08/2020
Interim Report #9	08/2021
Interim Report #10	08/2022
Study Completion:	08/2022
Final Report Submission:	08/2023

Submit the protocols to your IND 009900, with a cross-reference letter to this BLA. Submit the interim and final reports to your BLA. Prominently identify the submission with the following wording in bold capital letters at the top of the first page of the submission, as appropriate: **"Required Postmarketing Protocol Under 505(o)," "Required Postmarketing Final Report Under 505(o)," "Required Postmarketing Correspondence Under 505(o)."**

Section 505(o)(3)(E)(ii) of the FDCA requires you to report periodically on the status of any study or clinical trial required under this section. This section also requires you to periodically report to FDA on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Section 506B of the FDCA, as well as 21 CFR 601.70 requires you to report annually on the status of any postmarketing commitments or required studies or clinical trials.

FDA will consider the submission of your annual report under section 506B and 21 CFR 601.70 to satisfy the periodic reporting requirement under section 505(o)(3)(E)(ii) provided that you include the elements listed in 505(o) and 21 CFR 601.70. We remind you that to comply with 505(o), your annual report must also include a report on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Failure to submit an annual report for studies or clinical trials required under 505(o) on the date required will be considered a violation of FDCA section 505(o)(3)(E)(ii) and could result in enforcement action.

POSTMARKETING COMMITMENTS

POSTMARKETING COMMITMENTS NOT SUBJECT TO REPORTING REQUIREMENTS UNDER SECTION 506B

We remind you of the following postmarketing commitments:

2. Provide a plan for responding to potential pertuzumab shortages.

   The timetable you submitted by e-mail on June 8, 2012, states that you will complete this plan according to the following schedule:

Draft Plan Submission:	07/2012
Final Plan Submission:	09/2012

3. Conduct a stability study that includes real time and stressed stability testing to assess the stability of the drug substance manufactured from thaws #4 and #6 of the Q1/Q2 2012 pertuzumab campaign. Provide a root cause analysis relating to the cell bank issues. Submit the Final Report as a Prior Approval Supplement (PAS).

   The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	06/2012
Interim Report:	09/2012
Study Completion:	10/2014
Final Report:	12/2014

4. Conduct a process validation study to support manufacture of pertuzumab from the Master Cell Bank. Submit the Final Report as a PAS.

   The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

Study Completion:	12/2012
Final Report Submission:	02/2013

5. Conduct a process validation study to support manufacture of pertuzumab from a new Working Cell Bank. Submit the Final Report as a PAS.

   The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	04/2013
Study Completion:	09/2014
Final Report Submission:	10/2014

6. Conduct process validation studies to support manufacture of pertuzumab from Working Cell Banks by a modified process. Submit the Final Report as a PAS.

   The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	04/2014
Study Completion:	10/2015
Final Report Submission:	11/2015

7. Conduct stability studies of the Master Cell Bank at more frequent intervals than the currently proposed 10 years. Submit Interim Reports every four years and the Final Report after 20 years.

   The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	09/2012
Interim Report 1:	06/2016
Interim Report 2:	06/2020
Interim Report 3:	06/2024
Interim Report 4:	06/2028
Final Report Submission:	06/2032

8. Reassess release and stability specifications for pertuzumab drug substance and drug product through June 30, 2014. Submit the Final Report as a Changes Being Effected-30 Supplement (CBE-30).

   The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

Study Completion:	12/2014
Final Report Submission:	03/2015

9. Conduct a study to assess the ability of a non-reduced CE-SDS assay to detect and quantitate pertuzumab fragmentation. Submit the Final Report as a CBE-30.

   The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	09/2012
Study Completion:	07/2013
Final Report Submission:	09/2013

10. Conduct a study to establish a drug substance release specification to control for antibody-dependent cellular cytotoxicity (ADCC) activity of pertuzumab. Submit the Final Report as a PAS.

    The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

Study Completion:	02/2013
Final Report Submission:	03/2013

11. Conduct a study using end of production cells from commercial scale manufacturing that tests for *in vivo* adventitious viruses and genetic consistency. Submit the Final Report as a PAS.

    The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	08/2012
Study Completion:	12/2012
Final Report Submission:	02/2013

12. Re-qualify the bioburden test for the bulk drug substance and in-process bioburden samples. Submit the Final Report as a Changes Being Effected-0 Supplement (CBE-0).

    The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	06/2012
Study Completion:	07/2012
Final Report Submission:	12/2012

13. Revalidate the hold time for non-sterile cell culture media. Submit the Final Report as CBE-30.

    The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	04/2013
Study Completion:	08/2013
Final Report Submission:	12/2013

14. Conduct a comprehensive risk assessment regarding the microbial control of the cell culture process and generate an action plan based on the assessment. Submit the Final Report as CBE-30.

The timetable you submitted by e-mail on June 8, 2012, states that you will conduct this study according to the following schedule:

Final Protocol Submission:	09/2012
Final Report Submission:	03/2013

POSTMARKETING COMMITMENTS SUBJECT TO REPORTING REQUIREMENTS UNDER SECTION 506B

We remind you of the following additional postmarketing commitments:

15. Conduct a clinical trial to test whether the addition of hormonal therapy increases the efficacy of pertuzumab-based therapy in the hormone receptor-positive, HER2-positive metastatic breast cancer population. Study MO27775 (PERTAIN) as designed will be completed to fulfill this post-marketing commitment.

    The timetable you submitted on May 16, 2012, states that you will conduct this trial according to the following schedule:

Final Protocol Submission:	08/2012
Trial Completion:	09/2016
Final Report Submission:	03/2017

16. Submit the results of the final overall survival (OS) analysis of trial WO20698/TOC4129g as defined in your protocol Statistical Analysis Plan (SAP).

    The timetable you submitted on May 16, 2012, states that you will conduct this trial according to the following schedule:

Trial Completion:	12/2013
Final Report Submission:	05/2014

For all postmarketing commitments, submit clinical protocols to your IND 009900 for this product. Submit nonclinical and chemistry, manufacturing, and controls protocols and all final reports to this BLA. In addition, under 21 CFR 601.70 you should include a status summary of each commitment in your annual progress report of postmarketing studies to this BLA. The status summary should include expected summary completion and final report submission dates, any changes in plans since the last annual report, and, for clinical studies/trials, number of patients entered into each study/trial. All submissions, including supplements, relating to these postmarketing commitments should be prominently labeled "**Postmarketing Commitment Protocol**," "**Postmarketing Commitment Final Report**," or "**Postmarketing Commitment Correspondence**."

REQUIRED PEDIATRIC ASSESSMENTS

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients, new indications, new dosage forms, new dosing regimens, or new routes of administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication(s) in pediatric patients unless this requirement is waived, deferred, or inapplicable.

We are waiving the pediatric study requirement for this application in all pediatric age groups because the necessary studies are impossible or highly impracticable.

REPORTING REQUIREMENTS

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). You should submit postmarketing adverse experience reports to:

Food and Drug Administration
Center for Drug Evaluation and Research
Central Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at http://www.fda.gov/Safety/MedWatch/HowToReport/ucm166910.htm.

You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Compliance Risk Management and Surveillance
5901-B Ammendale Road
Beltsville, MD 20705-1266

Biological product deviations, sent by courier or overnight mail, should be addressed to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Compliance Risk Management and Surveillance
10903 New Hampshire Avenue, Bldg. 51, Room 4206
Silver Spring, MD 20903

CONTENT OF LABELING

As soon as possible, but no later than 14 days from the date of this letter, submit, via the FDA automated drug registration and listing system (eLIST), the content of labeling [21 601.14(b)] in structured product labeling (SPL) format, as described at http://www.fda.gov/ForIndustry/DataStandards/StructuredProductLabeling/default.htm, that is identical to the enclosed labeling text for the package insert. Information on submitting SPL files using eLIST may be found in the guidance for industry titled "SPL Standard for Content of Labeling Technical Qs and As" at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072392.pdf. For administrative purposes, please designate this submission "**Product Correspondence – Final SPL for approved BLA STN 125409/0.**"

The SPL will be accessible via publicly available labeling repositories.

CARTON AND IMMEDIATE CONTAINER LABELS

Submit final printed carton and container labels that are identical to the enclosed carton and immediate container labels as soon as they are available, but no more than 30 days after they are printed. Please submit these labels electronically according to the guidance for industry titled "Providing Regulatory Submissions in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008)". Alternatively, you may submit 12 paper copies, with 6 of the copies individually mounted on heavy-weight paper or similar material. For administrative purposes, designate this submission "**Product Correspondence – Final Printed Carton and Container Labels for approved BLA STN 125409/0**". Approval of this submission by FDA is not required before the labeling is used. Marketing the product(s) with final printed labeling (FPL) that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

PROMOTIONAL MATERIALS

You may request advisory comments on proposed introductory advertising and promotional labeling. To do so, submit, in triplicate, a cover letter requesting advisory comments, the proposed materials in draft or mock-up form with annotated references, and the package insert to:

Food and Drug Administration
Center for Drug Evaluation and Research
Office of Prescription Drug Promotion
5901-B Ammendale Road
Beltsville, MD 20705-1266

You must submit final promotional materials, and the package insert, at the time of initial dissemination or publication, accompanied by a Form FDA 2253. For instruction on completing the Form FDA 2253, see page 2 of the Form. For more information about submission of promotional materials to the Office of Prescription Drug Promotion (OPDP), see http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm090142.htm.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

POST-ACTION FEEDBACK MEETING

New molecular entities and new biologics qualify for a post-action feedback meeting. Such meetings are used to discuss the quality of the application and to evaluate the communication process during drug development and marketing application review. The purpose is to learn from successful aspects of the review process and to identify areas that could benefit from improvement. If you would like to have such a meeting with us, call the Regulatory Project Manager for this application.

If you have any questions, call Amy Tilley, Regulatory Project Manager, at (301) 796-3994.

Sincerely,

{See appended electronic signature page}

Richard Pazdur, M.D.
Director
Office of Hematology and Oncology Products
Center for Drug Evaluation and Research

ENCLOSURES:
Content of Labeling
Carton and Container Labeling

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

RICHARD PAZDUR
06/08/2012

Attachment C

U.S. Patent No. 6,949,245

US006949245B1

(12) **United States Patent**
Sliwkowski

(10) **Patent No.: US 6,949,245 B1**
(45) **Date of Patent: Sep. 27, 2005**

(54) **HUMANIZED ANTI-ERBB2 ANTIBODIES AND TREATMENT WITH ANTI-ERBB2 ANTIBODIES**

(75) Inventor: **Mark Sliwkowski**, San Carlos, CA (US)

(73) Assignee: **Genentech, Inc.**, South San Francisco, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 215 days.

(21) Appl. No.: **09/602,812**

(22) Filed: **Jun. 23, 2000**

Related U.S. Application Data

(60) Provisional application No. 60/141,316, filed on Jun. 25, 1999.

(51) **Int. Cl.**[7] **A61K 39/395**; C07K 16/28; C07K 16/30

(52) **U.S. Cl.** **424/143.1**; 424/130.1; 424/133.1; 424/135.1; 424/136.1; 424/138.1; 424/141.1; 424/142.1; 424/172.1; 424/174.1; 424/178.1; 424/800; 424/801

(58) **Field of Search** 424/143.1, 130.1, 424/133.1, 135.1, 136.1, 138.1, 141.1, 142.1, 172.1, 174.1, 178.1, 800, 801, 152.1, 155.1, 137.1

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Primary Examiner—Alana M. Harris
Assistant Examiner—Anne L. Holleran
(74) *Attorney, Agent, or Firm*—Wendy M. Lee

(57) **ABSTRACT**

The present application describes humanized anti-ErbB2 antibodies and methods for treating cancer with anti-ErbB2 antibodies, such as humanized anti-ErbB2 antibodies.

24 Claims, 14 Drawing Sheets

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  1 MELAALCRWG LLLALLPPGA ASTQVCTGTD MKLRLPASPE THLDMLRHLY QGCQVVQGNL ELTYLPTNAS LSFLQDIQEV QGYVLIAHNQ VRQVPLQRLR
101 IVRGTQLFED NYALAVLDNG DPLNNTTPVT GASPGGLREL QLRSLTEILK GGVLIQRNPQ LCYQDTILWK DIFHKNNQLA LTLIDTNRSR ACHPCSPMCK
201 GSRCWGESSE DCQSLTRTVC AGGCARCKGP LPTDCCHEQC AAGCTGPKHS DCLACLHFNH SGICELHCPA LVTYNTDTFE SMPNPEGRYT FGASCVTACP
301 YNYLSTDVGS CTLVCPLHNQ EVTAEDGTQR CEKCSKPCAR VCYGLGMEHL REVRAVTSAN IQEFAGCKKI FGSLAFLPES FDGDPASNTA PLQPEQLQVF
401 ETLEEITGYL YISAWPDSLP DLSVFQNLQV IRGRILHNGA YSLTLQGLGI SWLGLRSLRE LGSGLALIHH NTHLCFVHTV PWDQLFRNPH QALLHTANRP
501 EDECVGEGLA CHQLCARGHC WGPGPTQCVN CSQFLRGQEC VEECRVLQGL PREYVNARHC LPCHPECQPQ NGSVTCFGPE ADQCVACAHY KDPPFCVARC
601 PSGVKPDLSY MPIWKFPDEE GACQPCPINC THSCVDLDDK GCPAE (SEQ ID NO: 13)
```

Fig. 1A

7C2 aa 22-53 (31 residues)

7F3 aa 22-53 (31 residues)

2C4 aa 22-584 (562 residues)

7D3 aa 22-584 (562 residues)

3E8 aa 512-625 (113 residues)

4D5 aa 529-625 (96 residues)

2H11 aa 529-645 (116 residues)

3H4 aa 541-599 (58 residues)

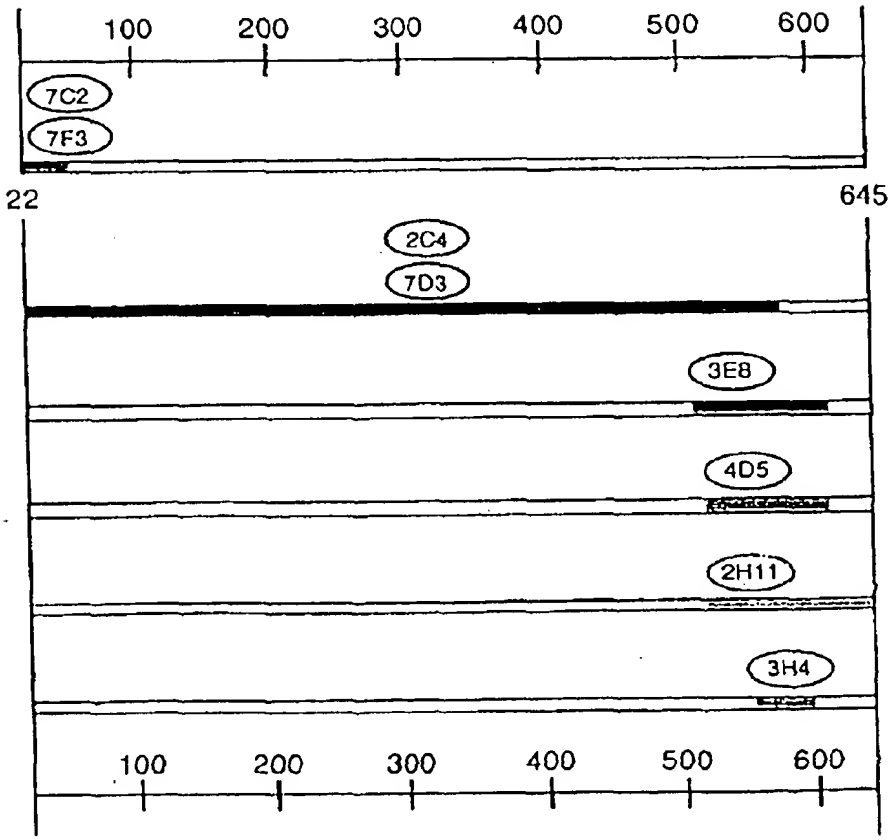


Fig. 1B

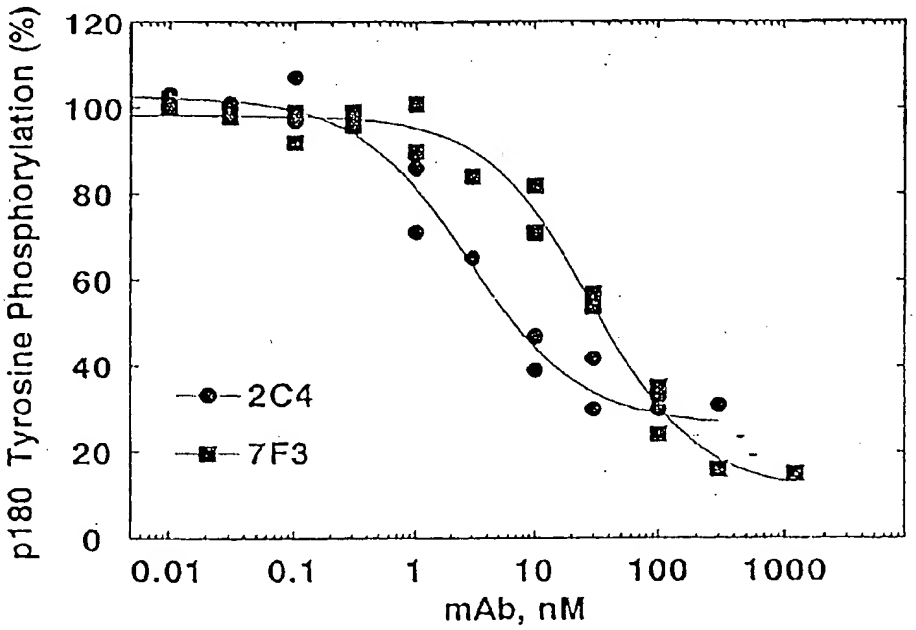

p180 Tyrosine Phosphorylation (%)
120
100
80
60
40
20
0
2C4
7F3
0.01
0.1
1
10
100
1000
mAb, nM

Fig. 2A

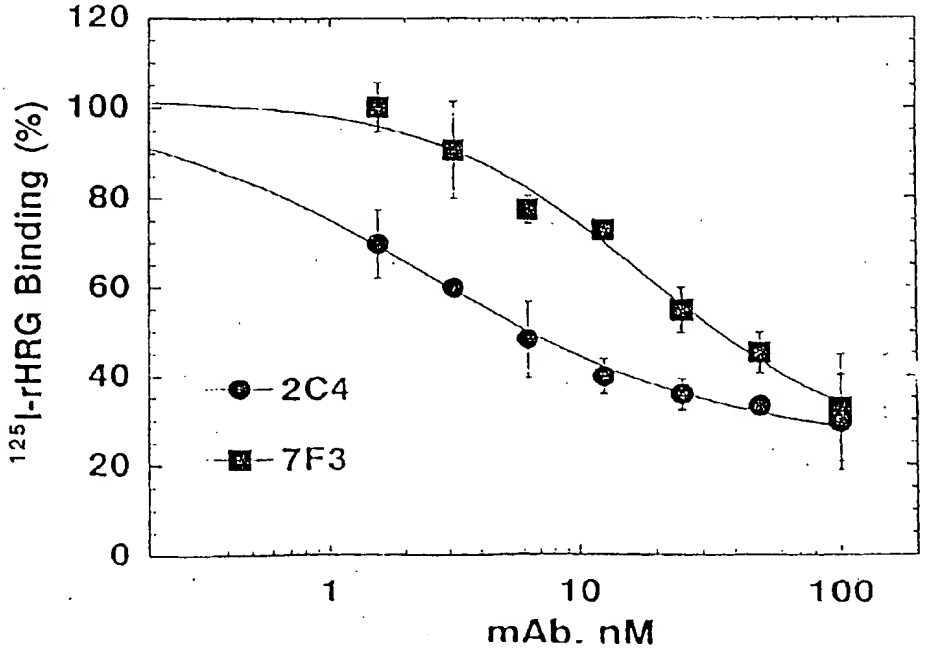

125I-rHRG Binding (%)
120
100
80
60
40
20
0
2C4
7F3
1
10
100
mAb. nM

Fig. 2B

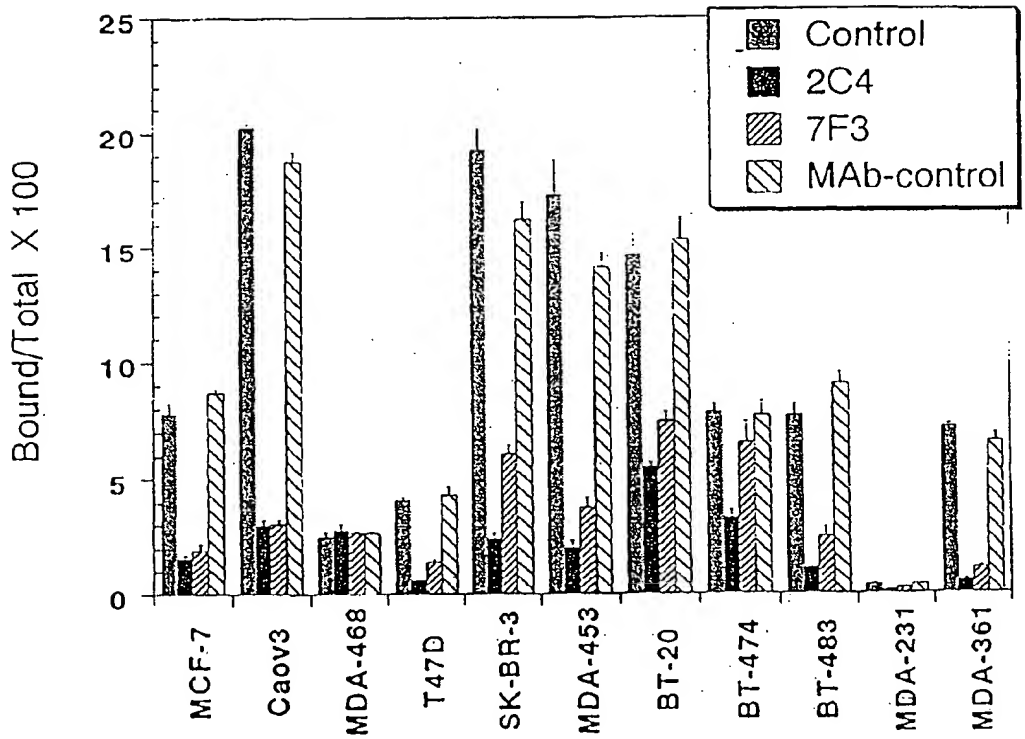

Bound/Total X 100
25
20
15
10
5
0
Control
2C4
7F3
MAb-control
MCF-7
Caov3
MDA-468
T47D
SK-BR-3
MDA-453
BT-20
BT-474
BT-483
MDA-231
MDA-361

Fig. 3

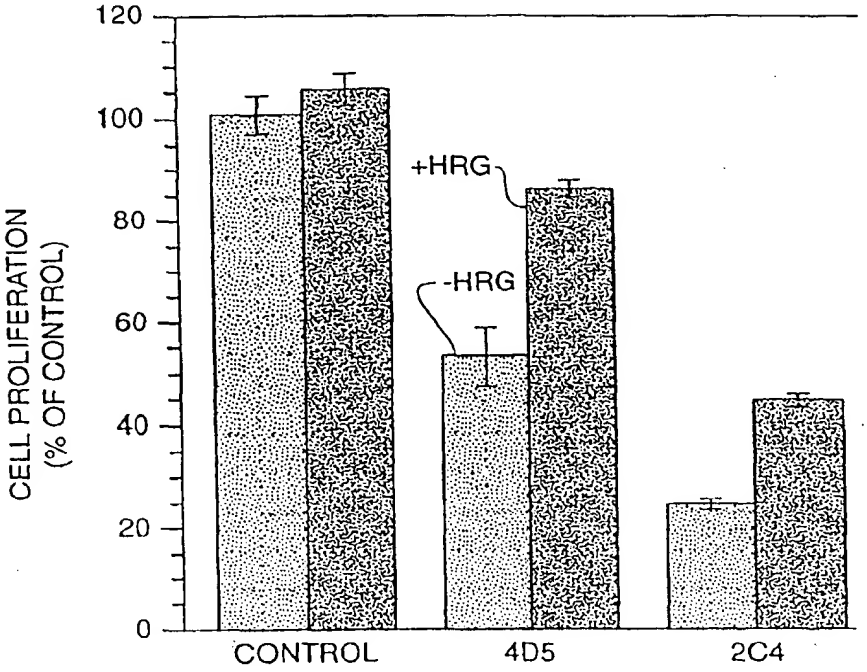

120
100
80
60
40
20
0
CELL PROLIFERATION
(% OF CONTROL)
+HRG
-HRG
CONTROL
4D5
2C4

Fig. 4A

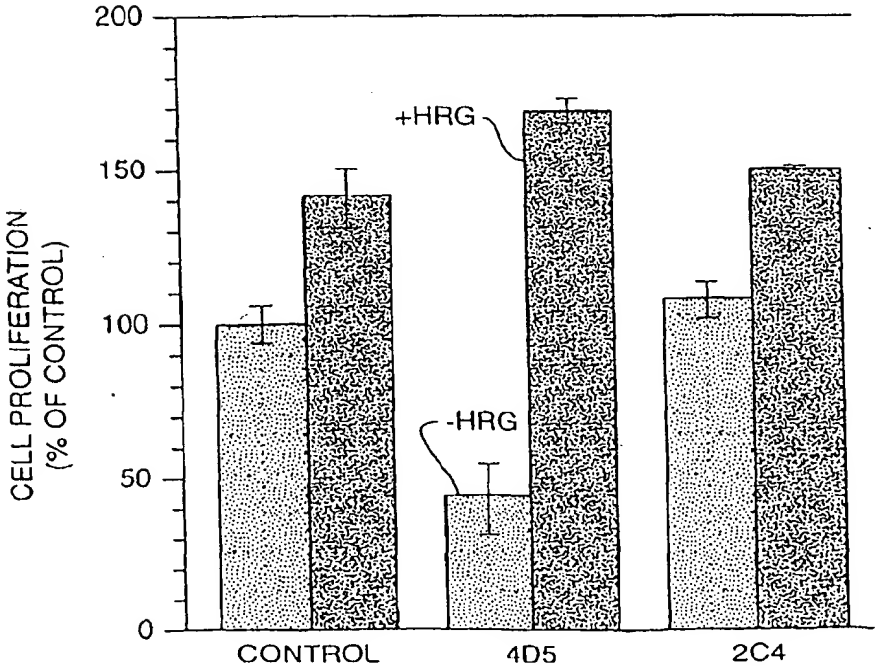

200
150
100
50
0
CELL PROLIFERATION
(% OF CONTROL)
+HRG
-HRG
CONTROL
4D5
2C4

Fig. 4B

Fig. 5A

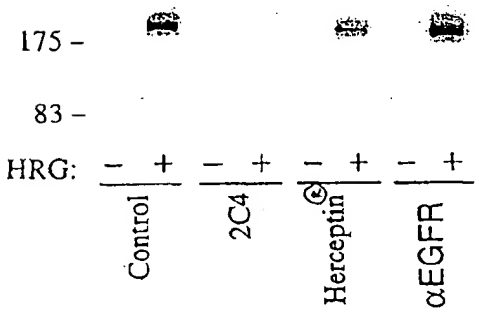
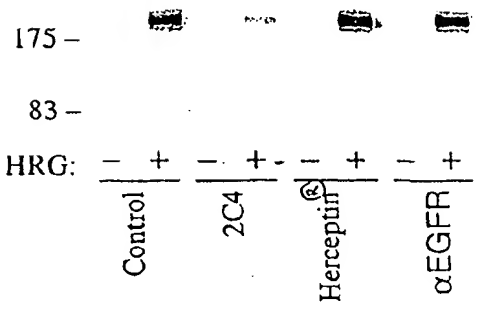


Fig. 5B



IP: αHER2
WB: αHER3

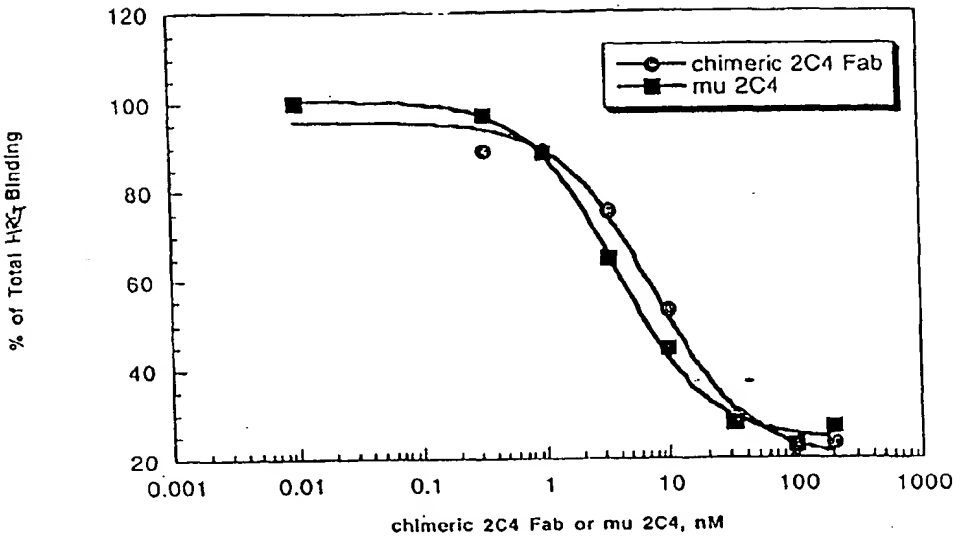

chimeric 2C4 Fab
mu 2C4
% of Total HRG Binding
120
100
80
60
40
20
0.001
0.01
0.1
1
10
100
1000
chimeric 2C4 Fab or mu 2C4, nM

Fig. 6A

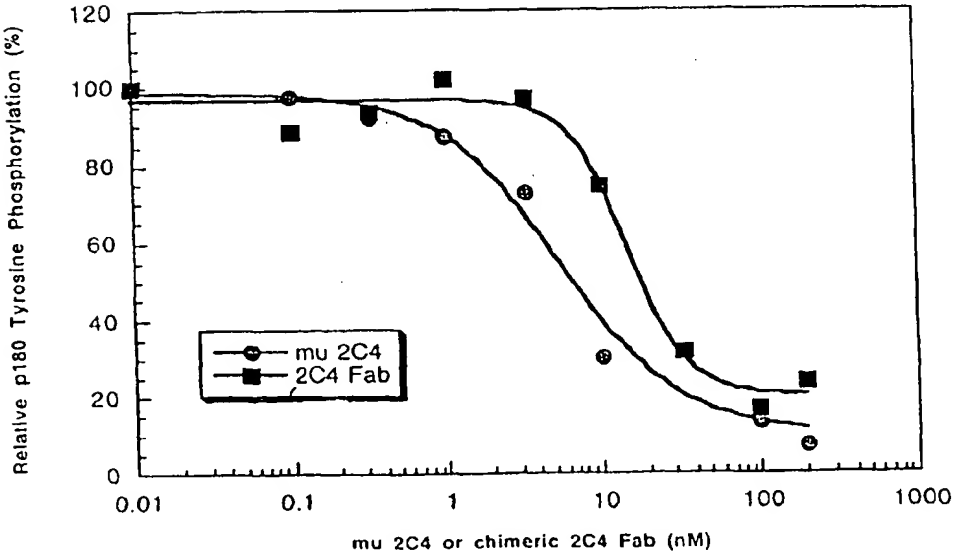

mu 2C4
2C4 Fab
Relative p180 Tyrosine Phosphorylation (%)
120
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
mu 2C4 or chimeric 2C4 Fab (nM)

Fig. 6B

Fig. 7A: Variable Light

```
                10        20          30          40
2C4     DTVMTQSHKIMSTSVGDRVSITC [KASQDVSIGVA] WYQQRP
         **    **** *      *                     *
574     DIQMTQSPSSLSASVGDRVTITC [KASQDVSIGVA] WYQQKP
                                  *   ** ***
hum κI  DIQMTQSPSSLSASVGDRVTITC [RASQSISNYLA] WYQQKP

                50          60          70          80
2C4     GQSPKLLIY [SASYRYT] GVPDRFTGSGSGTDFTFTISSVQA
          **                 *  *          *    * *
574     GKAPKLLIY [SASYRYT] GVPSRFSGSGSGTDFTLTISSLQP
                   * *****
hum κI  GKAPKLLIY [AASSLES] GVPSRFSGSGSGTDFTLTISSLQP

                90         100
2C4     EDLAVYYC [QQYYIYPYT] FGGGTKLEIK (SEQ ID NO:1)
          * *                 *   *
574     EDFATYYC [QQYYIYPYT] FGQGTKVEIK (SEQ ID NO:3)
                    *** *
hum κI  EDFATYYC [QQYNSLPWT] FGQGTKVEIK (SEQ ID NO:5)
```

Fig. 7B: Variable Heavy

```
                10        20          30          40
2C4     EVQLQQSGPELVKPGTSVKISCKAS [GFTFTDYTMD] WVKQS
          **  **  *  * *** *                    * *
574     EVQLVESGGGLVQPGGSLRLSCAAS [GFTFTDYTMD] WVRQA
                                    ** * *
hum III EVQLVESGGGLVQPGGSLRLSCAAS [GFTFSSYAMS] WVRQA

                50 a       60          70          80
2C4     HGKSLEWIG [DVNPNSGGSIYNQRFKG] KASLTVDRSSRIVYM
          *  *   **                   *** *    **** *
574     PGKGLEWVA [DVNPNSGGSIYNQRFKG] RFTLSVDRSKNTLYL
                   ****** *** ****       * * *
hum III PGKGLEWVA [VISGDGGSTYYADSVKG] RFTISRDNSKNTLYL

              abc      90         100ab        110
2C4     ELRSLTFEDTAVYYCAR [NLGPSFYFDY] WGQGTTLTVSS (SEQ ID NO:2)
          ***  **                                **
574     QMNSLRAEDTAVYYCAR [NLGPSFYFDY] WGQGTLVTVSS (SEQ ID NO:4)
                           ********
hum III QMNSLRAEDTAVYYCAR [GRVGYSLYDY] WGQGTLVTVSS (SEQ ID NO:6)
```

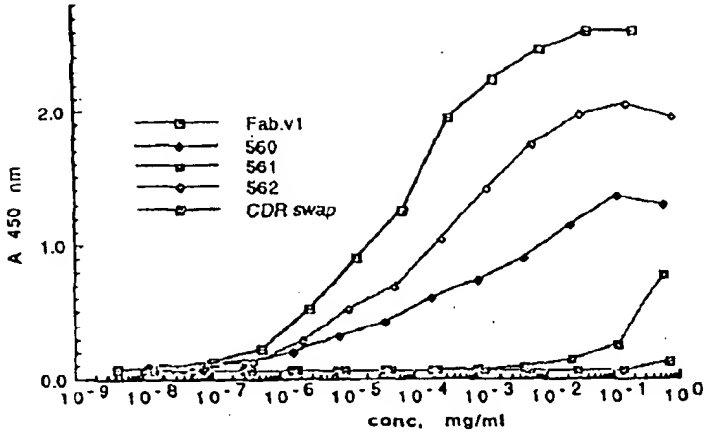


Fig. 8A

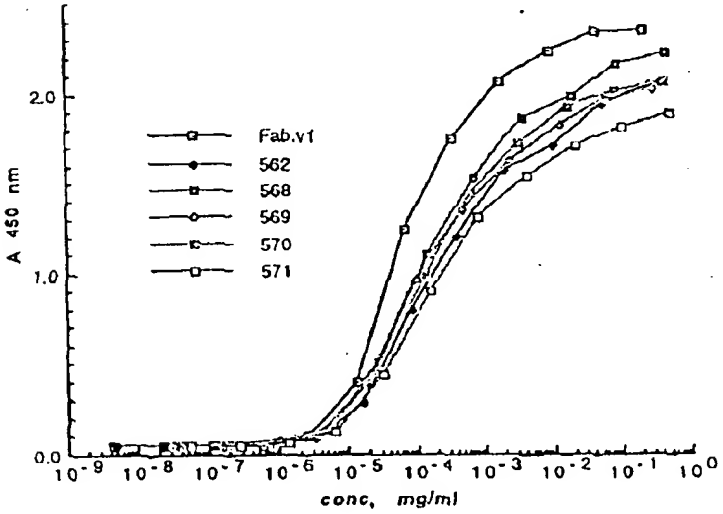


Fig. 8B

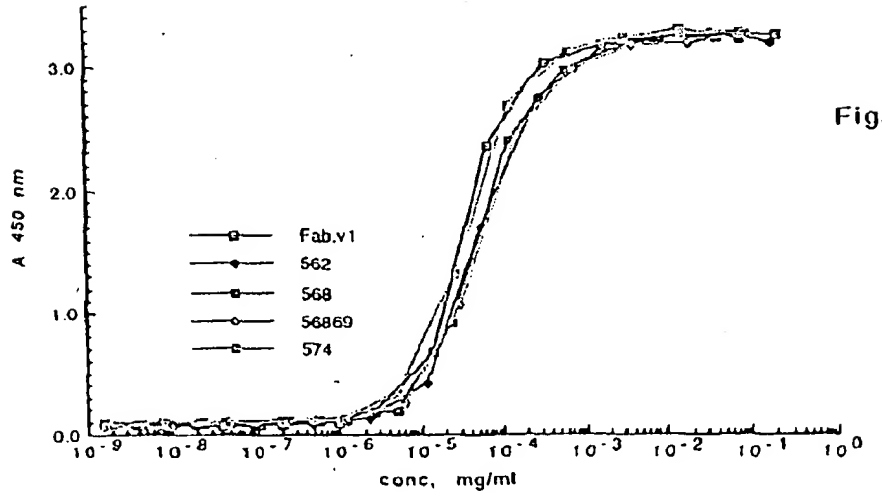


Fig. 8C

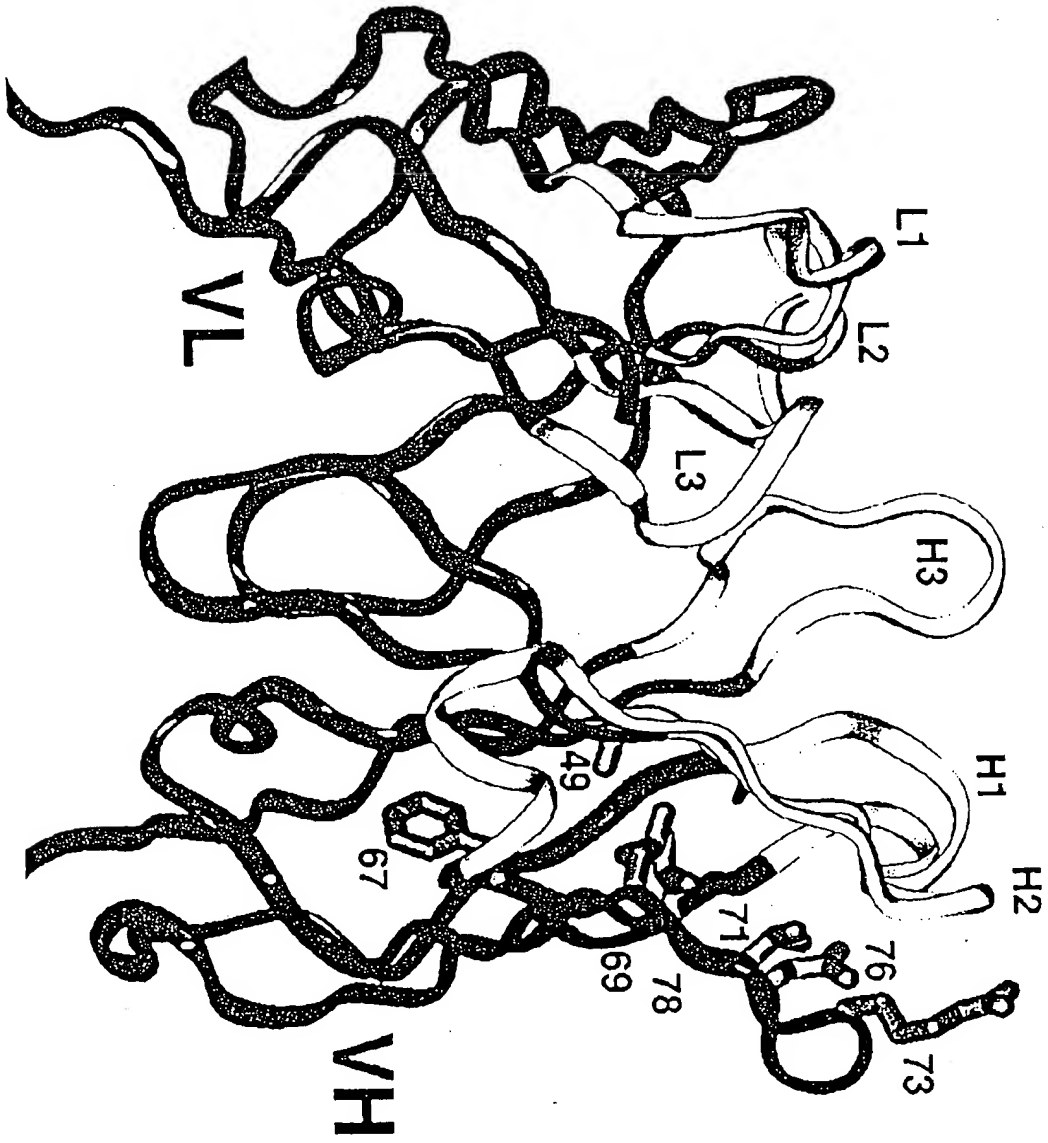
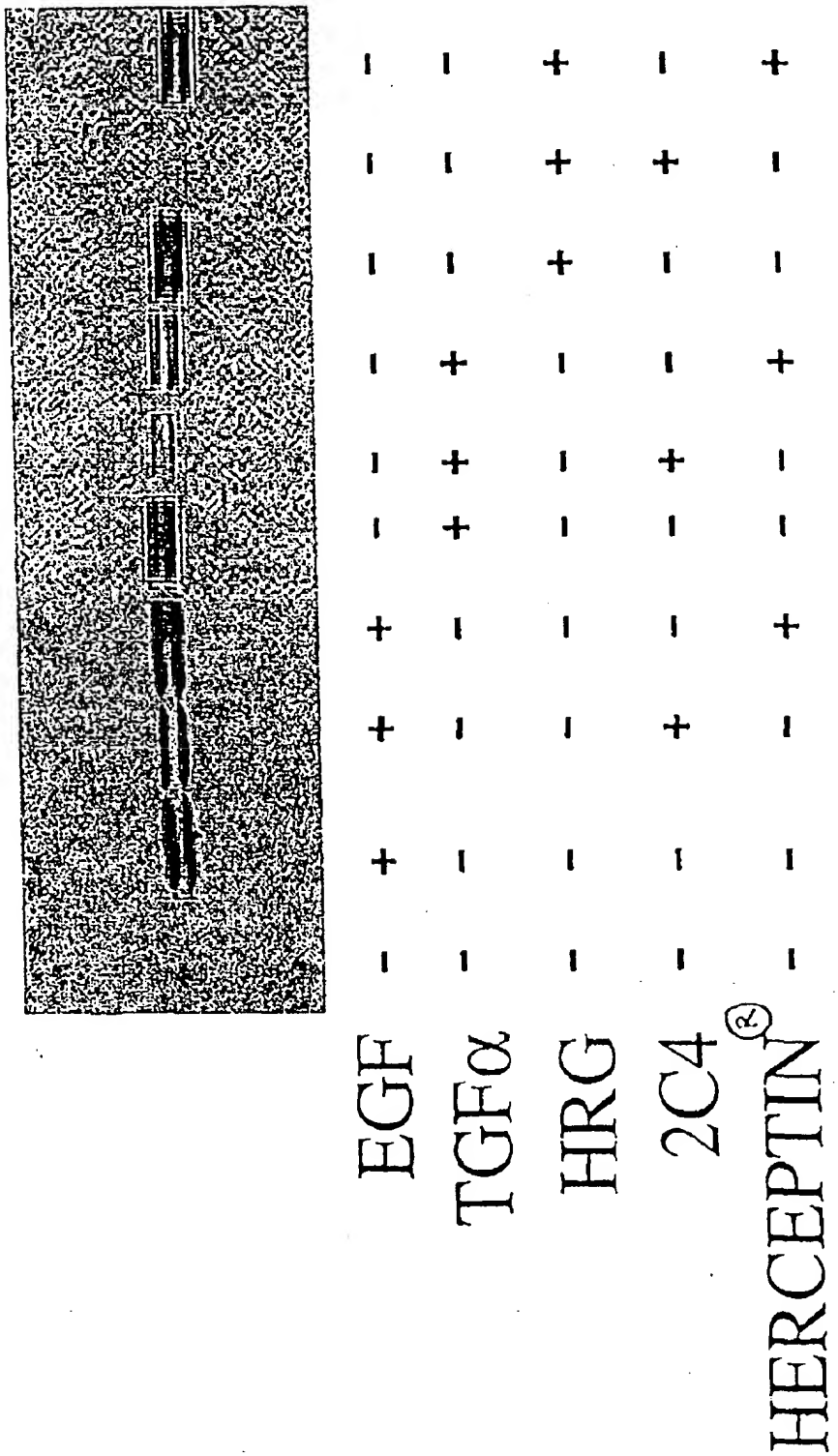


Fig. 9

Fig. 10



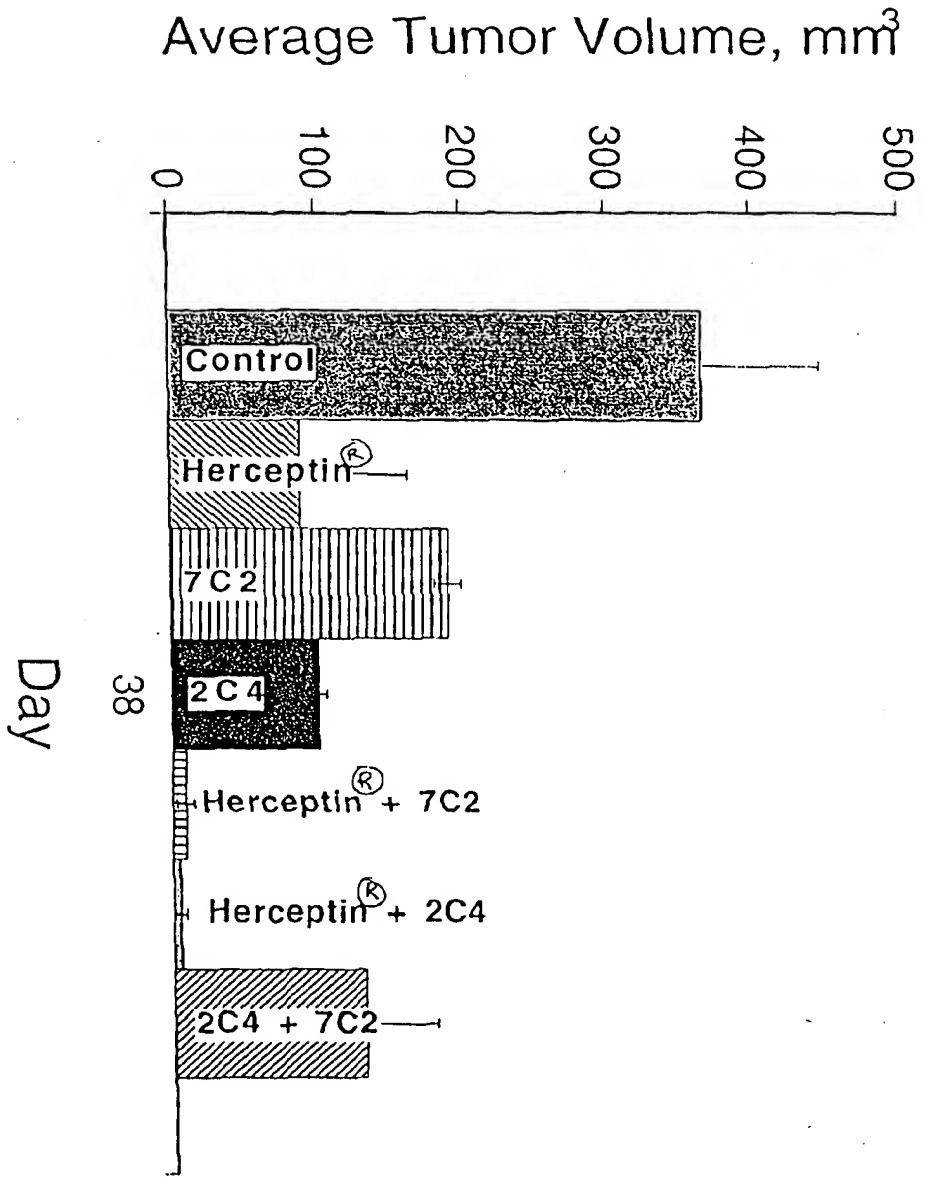

Average Tumor Volume, mm3
0
100
200
300
400
500
Control
Herceptin®
7C2
2C4
Herceptin® + 7C2
Herceptin® + 2C4
2C4 + 7C2
Day
38

Fig. 11

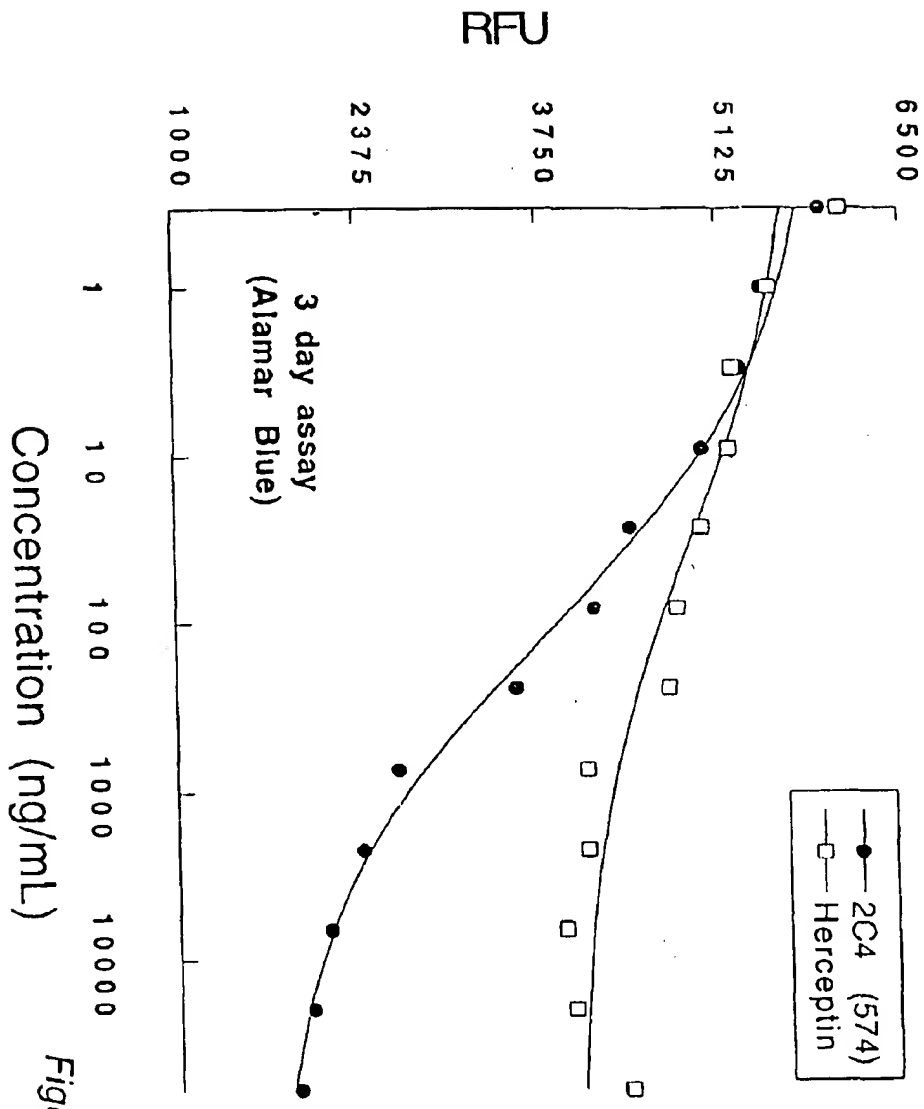

RFU
6500
5125
3750
2375
1000
3 day assay
(Alamar Blue)
2C4 (574)
Herceptin
1
10
100
1000
10000
Concentration (ng/mL)

Figure 12

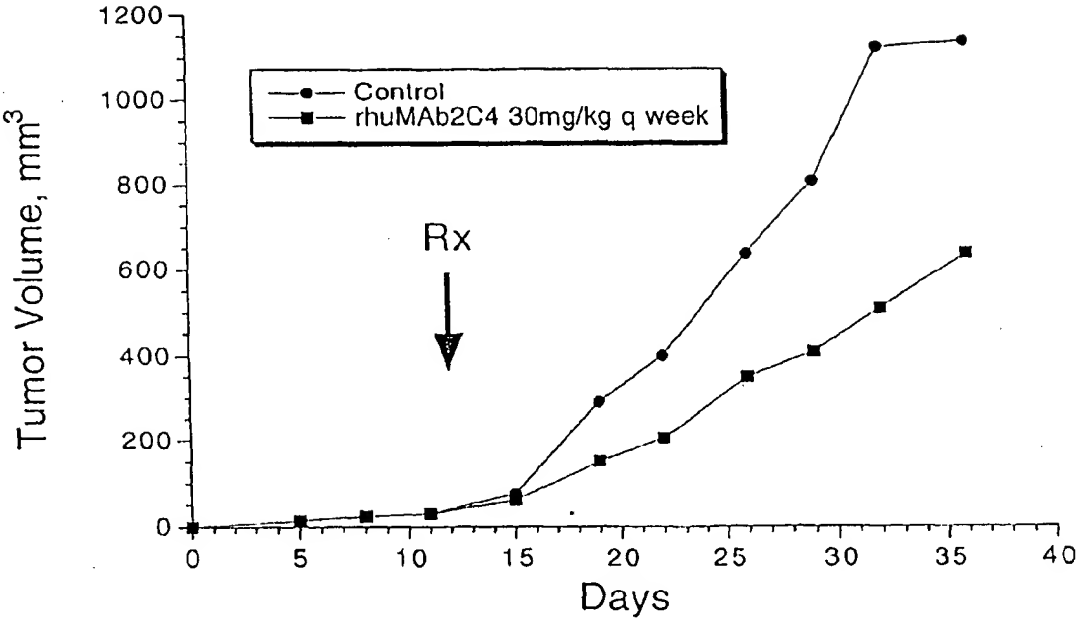

Tumor Volume, mm3
1200
1000
800
600
400
200
0
Control
rhuMAb2C4 30mg/kg q week
Rx
0
5
10
15
20
25
30
35
40
Days

Fig. 13

HUMANIZED ANTI-ERBB2 ANTIBODIES AND TREATMENT WITH ANTI-ERBB2 ANTIBODIES

RELATED APPLICATIONS

This application is a non-provisional application filed under 37 CFR 1.53(b)(1), claiming priority under 35 USC 119(e) to provisional application No. 60\141,316 filed Jun. 25, 1999, the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention concerns humanized anti-ErbB2 antibodies and methods for treating cancer with anti-ErbB2 antibodies, such as humanized anti-ErbB2 antibodies.

BACKGROUND OF THE INVENTION

The ErbB family of receptor tyrosine kinases are important mediators of cell growth, differentiation and survival. The receptor family includes four distinct members including epidermal growth factor receptor (EGFR or ErbB1), HER2 (ErbB2 or p185^{neu}), HER3 (ErbB3) and HER4 (ErbB4 or tyro2).

EGFR, encoded by the erbB1 gene, has been causally implicated in human malignancy. In particular, increased expression of EGFR has been observed in breast, bladder, lung, head, neck and stomach cancer as well as glioblastomas. Increased EGFR receptor expression is often associated with increased production of the EGFR ligand, transforming growth factor alpha (TGF-α), by the same tumor cells resulting in receptor activation by an autocrine stimulatory pathway. Baselga and Mendelsohn *Pharmac. Ther.* 64:127–154 (1994). Monoclonal antibodies directed against the EGFR or its ligands, TGF-α and EGF, have been evaluated as therapeutic agents in the treatment of such malignancies. See, e.g., Baselga and Mendelsohn., supra; Masui et al. *Cancer Research.* 44:1002–1007 (1984); and Wu et al. *J. Clin. Invest.* 95:1897–1905 (1995).

The second member of the ErbB family, p185^{neu}, was originally identified as the product of the transforming gene from neuroblastomas of chemically treated rats. The activated form of the neu proto-oncogene results from a point mutation (valine to glutamic acid) in the transmembrane region of the encoded protein. Amplification of the human homolog of neu is observed in breast and ovarian cancers and correlates with a poor prognosis (Slamon et al., *Science,* 235:177–182 (1987); Slamon et al., *Science,* 244:707–712 (1989); and U.S. Pat. No. 4,968,603). To date, no point mutation analogous to that in the neu proto-oncogene has been reported for human tumors. Overexpression of ErbB2 (frequently but not uniformly due to gene amplification) has also been observed in other carcinomas including carcinomas of the stomach, endometrium, salivary gland, lung, kidney, colon, thyroid, pancreas and bladder. See, among others, King et al., *Science,* 229:974 (1985); Yokota et al., *Lancet:* 1:765–767 (1986); Fukushige et al., *Mol Cell Biol.,* 6:955–958 (1986); Guerin et al., *Oncogene Res.,* 3:21–31 (1988); Cohen et al., *Oncogene,* 4:81–88 (1989); Yonemura et al., *Cancer Res.,* 51:1034 (1991); Borst et al., *Gynecol. Oncol.,* 38:364 (1990); Weiner et al., *Cancer Res.,* 50:421–425 (1990); Kern et al., *Cancer Res.,* 50:5184 (1990); Park et al., *Cancer Res.,* 49:6605 (1989); Zhau et al., *Mol. Carcinog.,* 3:254–257 (1990); Aasland et al. *Br. J. Cancer* 57:358–363 (1988); Williams et al. Pathobiology 59:46–52 (1991); and McCann et al., *Cancer,* 65:88–92 (1990). ErbB2 may be overexpressed in prostate cancer (Gu et al. *Cancer Lett.* 99:185–9 (1996); Ross et al. *Hum. Pathol.* 28:827–33 (1997); Ross et al. *Cancer* 79:2162–70 (1997); and Sadasivan et al. *J. Urol.* 150:126–31 (1993)).

Antibodies directed against the rat p185^{neu} and human ErbB2 protein produces have been described. Drebin and colleagues have raised antibodies against the rat neu gene product, p185^{neu} See, for example, Drebin et al., *Cell* 41:695–706 (1985); Myers et al., *Meth. Enzym.* 198:277–290 (1991); and WO94/22478. Drebin et al. *Oncogene* 2:273–277 (1988) report that mixtures of antibodies reactive with two distinct regions of p185^{neu} result in synergistic anti-tumor effects on neu-transformed NIH-3T3 cells implanted into nude mice. See also U.S. Pat. No. 5,824,311 issued Oct. 20, 1998.

Hudziak et al., *Mol. Cell. Biol.* 9(3):1165–1172 (1989) describe the generation of a panel of anti-ErbB2 antibodies which were characterized using the human breast tumor cell line SK-BR-3. Relative cell proliferation of the SK-BR-3 cells following exposure to the antibodies was determined by crystal violet staining of the monolayers after 72 hours. Using this assay, maximum inhibition was obtained with the antibody called 4D5 which inhibited cellular proliferation by 56%. Other antibodies in the panel reduced cellular proliferation to a lesser extent in this assay. The antibody 4D5 was further found to sensitize ErbB2-overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α. See also U.S. Pat. No. 5,677,171 issued Oct. 14, 1997. The anti-ErbB2 antibodies discussed in Hudziak et al are further characterized in Fendly et al *Cancer Research* 50:1550–1558 (1990); Kotts et al. In Vitro 26(3):59A (1990); Sarup et al. *Growth Regulation* 1:72–82 (1991); Shepard et al *J. Clin. Immunol.* 11(3):117–127 (1991); Kumar et al. *Mol. Cell. Biol.* 11(2):979–986 (1991); Lewis et al *Cancer Immunol. Immunother.* 37:255–263 (1993); Pietras et al *Oncogene* 9:1829–1838 (1994); Vitetta et al *Cancer Research* 54:5301–5309 (1994); Sliwkowski et al. *J. Biol. Chem.* 269(20):14661–14665 (1994); Scott et al. *J. Biol. Chem.* 266:14300–5 (1991); D'souza et al *Proc. Natl. Acad. Sci.* 91:7202–7206 (1994); Lewis et al. *Cancer Research* 56:1457–1465 (1996); and Schaefer et al. *Oncogene* 15:1385–1394 (1997).

A recombinant humanized version of the murine anti-ErbB2 antibody 4D5 (huMAb4D5-8, rhuMAb HER2 or HERCEPTIN®; U.S. Pat. No. 5,821,337) is clinically active in patients with ErbB2-overexpressing metastatic breast cancers that have received extensive prior anticancer therapy (Baselga et al., *J. Clin. Oncol.* 14:737–744 (1996)). HERCEPTIN® received marketing approval from the Food and Drug Administration Sep. 25, 1998 for the treatment of patients with metastatic breast cancer whose tumors overexpress the ErbB2 protein.

Other anti-ErbB2 antibodies with various properties have been described in Tagliabue et al. *Int. J. Cancer* 47:933–937 (1991); McKenzie et al. *Oncogene* 4:543–548 (1989); Maier et al. *Cancer Res.* 51:5361–5369 (1991); Bacus et al. *Molecular Carcinogenesis* 3:350–362 (1990); Stancovski et al. *PNAS* (USA) 88:8691–8695 (1991); Bacus et al. *Cancer Research* 52:2580–2589 (1992); Xu et al *Int. J. Cancer* 53:401–408 (1993); WO94/00136; Kasprzyk et al. *Cancer Research* 52:2771–2776 (1992); Hancock et al. *Cancer Res.* 51:4575–4580 (1991); Shawver et al. *Cancer Res.* 54:1367–1373(1994); Arteaga et al. *Cancer Res.* 54:3758–3765 (1994); Harwerth et al. *J. Biol. Chem.* 267:15160–15167 (1992); U.S. Pat. No. 5,783,186; and Klapper et al. *Oncogene* 14:2099–2109 (1997).

Homology screening has resulted in the identification of two other ErbB receptor family members; ErbB3 (U.S. Pat.

Nos. 5,183,884 and 5,480,968 as well as Kraus et al. *PNAS* (USA) 86:9193–9197 (1989)) and ErbB4 (EP Pat Appln No 599,274; Plowman et al., *Proc. Nail. Acad. Sci. USA,* 90:1746–1750 (1993); and Plowman et al, *Nature,* 366:473–475 (1993)). Both of these receptors display increased expression on at least some breast cancer cell lines.

The ErbB receptors are generally found in various combinations in cells and heterodimerization is thought to increase the diversity of cellular responses to a variety of ErbB ligands (Earp et al. *Breast Cancer Research and Treatment* 35: 115–132 (1995)). EGFR is bound by six different ligands; epidermal growth factor (EGF), transforming growth factor alpha (TGF-α), amphiregulin, heparin binding epidermal growth factor (HB-EGF), betacellulin and epiregulin (Groenen et al. *Growth Factors* 11:235–257 (1994)). A family of heregulin proteins resulting from alternative splicing of a single gene are ligands for ErbB3 and ErbB4. The heregulin family includes alpha, beta and gamma heregulins (Holmes et al., *Science,* 256:1205–1210 (1992); U.S. Pat. No. 5,641,869; and Schaefer et al. *Oncogene* 15:1385–1394 (1997)); neu differentiation factors (NDFs), glial growth factors (GGFs); acetylcholine receptor inducing activity (ARIA); and sensory and motor neuron derived factor (SMDF). For a review, see Groenen et al. *Growth Factors* 11:235–257 (1994); Lemke, G. *Molec. & Cell. Neurosci.* 7:247–262 (1996) and Lee et al. *Pharm. Rev.* 47:51–85 (1995). Recently three additional ErbB ligands were identified; neuregulin-2 (NRG-2) which is reported to bind either ErbB3 or ErbB4 (Chang et al. *Nature* 387 509–512 (1997); and Carraway et al *Nature* 387:512–516 (1997)); neuregulin-3 which binds ErbB4 (Zhang et al. *PNAS* (USA) 94(18):9562–7 (1997)); and neuregulin-4 which binds ErbB4 (Harari et al. *Oncogene* 18:2681–89 (1999)) HB-EGF, betacellulin and epiregulin also bind to ErbB4.

While EGF and TGFα do not bind ErbB2, EGF stimulates EGFR and ErbB2 to form a heterodimer, which activates EGFR and results in transphosphorylation of ErbB2 in the heterodimer. Dimerization and/or transphosphorylation appears to activate the ErbB2 tyrosine kinase. See Earp et al., supra. Likewise, when ErbB3 is co-expressed with ErbB2, an active signaling complex is formed and antibodies directed against ErbB2 are capable of disrupting this complex (Sliwkowski et al., *J. Biol. Chem.,* 269(20): 14661–14665 (1994)). Additionally, the affinity of ErbB3 for heregulin (HRG) is increased to a higher affinity state when co-expressed with ErbB2. See also, Levi et al., *Journal of Neuroscience* 15: 1329–1340 (1995); Morrissey et al., *Proc. Nat. Acad. Sci. USA* 92: 1431–1435 (1995); and Lewis et al., *Cancer Res.,* 56:1457–1465 (1996) with respect to the ErbB2–ErbB3 protein complex. ErbB4, like ErbB3, forms an active signaling complex with ErbB2 (Carraway and Cantley, *Cell* 78:5–8 (1994)).

SUMMARY OR THE INVENTION

In a first aspect, the present invention provides a method of treating cancer in a human, wherein the cancer expresses epidermal growth factor receptor (EGFR), comprising administering to the human a therapeutically effective amount of an antibody which binds ErbB2.

Various advantages in using an antibody which binds ErbB2 to treat such cancer, as opposed to EGFR-targeted drugs, are contemplated herein. In particular, EGFR is highly expressed in liver and skin and this provides an enormous sink for active drug where the drug binds to EGFR. In addition, skin toxicity has been observed for other EGFR-targeted drugs such as the chimeric anti-EGFR antibody C225 and the small molecule drug ZD1839 which binds EGFR. Antibodies which bind ErbB2 are anticipated to have a better safety profile than such drugs.

Where the antibody used for therapy herein blocks ligand activation of an ErbB receptor and/or has a biological characteristic of monoclonal antibody 2C4, further advantages are achieved. For example, while EGFR-targeted drugs interfere only with EGFR, the antibodies of particular interest herein (e.g. 2C4, including humanized and/or affinity matured variants thereof) will interfere with EGFR/ErbB2, ErbB3/ErbB4 and ErbB2/ErbB3 heterodimers. In addition, the antibodies herein that bind ErbB2 and block ligand activation of an ErbB receptor will be complementary to EGFR-targeted drugs, where EGFR-targeted drugs are not complementary to each other.

The invention further provides a method of treating cancer in a human, wherein the cancer is not characterized by overexpression of the ErbB2 receptor, comprising administering to the human a therapeutically effective amount of an antibody which binds to ErbB2 and blocks ligand activation of an ErbB receptor.

In addition, the present invention provides a method of treating hormone independent cancer in a human comprising administering to the human a therapeutically effective amount of an antibody which binds ErbB2 receptor, and blocks ligand activation of an ErbB receptor.

The invention further provides a method of treating cancer in a human comprising administering to the human therapeutically effective amounts of (a) a first antibody which binds ErbB2 and inhibits growth of cancer cells which overexpress ErbB2; and (b) a second antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor.

The invention also provides a method of treating a cancer in a human, wherein the cancer is selected from the group consisting of colon, rectal and colorectal cancer, comprising administering to the human a therapeutically effective amount of an antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor.

In further embodiments, the invention provides articles of manufacture for use (among other things) in the above methods. For example, the invention provides an article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an antibody which binds ErbB2, and further comprising a package insert indicating that the composition can be used to treat cancer which expresses epidermal growth factor receptor (EGFR).

The invention additionally pertains to an article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor, and further comprising a package insert indicating that the composition can be used to treat cancer, wherein the cancer is not characterized by overexpression of the ErbB2 receptor.

Also, the invention relates to an article of manufacture comprising a container and a composition contained therein, wherein the composition comprises an antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor, and further comprising a package insert indicating that the composition can be used to treat hormone independent cancer.

In a further embodiment, an article of manufacture is provided which comprises (a) a first container with a com-

position contained therein, wherein the composition comprises a first antibody which binds ErbB2 and inhibits growth of cancer cells which overexpress ErbB2; and (b) a second container with a composition contained therein, wherein the composition comprises a second antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor.

A further article of manufacture is provided which comprises a container and a composition contained therein, wherein the composition comprises an antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor, and further comprises a package insert indicating that the composition can be used to treat a cancer selected from the group consisting of colon, rectal and colorectal cancer.

The invention additionally provides: a humanized antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor; a composition comprising the humanized antibody and a pharmaceutically acceptable carrier; and an immunoconjugate comprising the humanized antibody conjugated with a cytotoxic agent.

Moreover, the invention provides isolated nucleic acid encoding the humanized antibody; a vector comprising the nucleic acid; a host cell comprising the nucleic acid or the vector; as well as a process of producing the humanized antibody comprising culturing a host cell comprising the nucleic acid so that the nucleic acid is expressed and, optionally, further comprising recovering the humanized antibody from the host cell culture (e.g. from the host cell culture medium).

The invention further pertains to an immunoconjugate comprising an antibody which binds ErbB2 conjugated to one or more calicheamicin molecules, and the use of such conjugates for treating ErbB2 expressing cancer, e.g., ErbB2 overexpressing cancer, in a human. Preferably, the antibody in the conjugate is monoclonal antibody 4D5, e.g., humanized 4D5 (and preferably huMAb4D5-8 (HERCEPTIN®); or monoclonal antibody 2C4, e.g., humanized 2C4. The antibody in the immunoconjugate may be an intact antibody (e.g., an intact IgG_1 antibody) or an antibody fragment (e.g. a Fab, $F(ab)_2$, diabody etc).

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A and 1B depict epitope mapping of residues 22–645 within the extracellular domain (ECD) of ErbB2 (amino acid sequence, including signal sequence, shown in FIG. 1A; SEQ ID NO:13) as determined by truncation mutant analysis and site-directed mutagenesis (Nakamura et al. *J. of Virology* 67(10):6179–6191 (1993); and Renz et al. *J. Cell Biol.* 125(6): 1395–1406 (1994)). The various ErbB2-ECD truncations or point mutations were prepared from cDNA using polymerase chain reaction technology. The ErbB2 mutants were expressed as gD fusion proteins in a mammalian expression plasmid. This expression plasmid uses the cytomegalovirus promoter/enhancer with SV40 termination and polyadenylation signals located downstream of the inserted cDNA. Plasmid DNA was transfected into 293 cells. One day following transfection, the cells were metabolically labeled overnight in methionine and cysteine-free, low glucose DMEM containing 1% dialyzed fetal bovine serum and 25 μCi each of ^{35}S methionine and ^{35}S cysteine. Supernatants were harvested and either the anti-ErbB2 monoclonal antibodies or control antibodies were added to the supernatant and incubated 24 hours at 4° C. The complexes were precipitated, applied to a 10–20% Tricine SDS gradient gel and electrophoresed at 100 V. The gel was electroblotted onto a membrane and analyzed by autoradiography. As shown in FIG. 1B, the anti-ErbB2 antibodies 7C2, 7F3, 2C4, 7D3, 3E8, 4D5, 2H11 and 3H4 bind various ErbB2 ECD epitopes.

FIGS. 2A and 2B show the effect of anti-ErbB2 monoclonal antibodies 2C4 and 7F3 on rHRGβ1 activation of MCF7 cells. FIG. 2A shows dose-response curves for 2C4 or 7F3 inhibition of HRG stimulation of tyrosine phosphorylation. FIG. 2B shows dose-response curves for the inhibition of ^{125}I-labeled $rHRG\beta1_{177-244}$ binding to MCF7 cells by 2C4 or 7F3.

FIG. 3 depicts inhibition of specific ^{125}I-labeled $rHRG\beta1_{177-244}$ binding to a panel of human tumor cell lines by the anti-ErbB2 monoclonal antibodies 2C4 or 7F3. Monoclonal antibody-controls are isotype-matched murine monoclonal antibodies that do not block rHRG binding. Nonspecific ^{125}I-labeled $rHR\beta1_{1177-244}$ binding was determined from parallel incubations performed in the presence of 100 nM rHRGβ1. Values for nonspecific ^{125}I-labeled $rHRG\beta1_{177-244}$ binding were less than 1% of the total for all the cell lines tested.

FIGS. 4A and 4B show the effect of monoclonal antibodies 2C4 and 4D5 on proliferation of MDA-MB-175 (FIG. 4A) and SK-BR-3 (FIG. 4B) cells. MDA-MB-175 and SK-BR-3 cells were seeded in 96 well plates and allowed to adhere for 2 hours. Experiment was carried out in medium containing 1% serum. Anti-ErbB2 antibodies or medium alone were added and the cells were incubated for 2 hours at 37° C. Subsequently rHRGβ1(1 nM) or medium alone were added and the cells were incubated for 4 days. Monolayers were washed and stained/fixed with 0.5% crystal violet. To determine cell proliferation the absorbance was measured at 540 nm.

FIGS. 5A and 5B show the effect of monoclonal antibody 2C4, HERCEPTIN® antibody or an anti-EGFR antibody on heregulin (HRG) dependent association of ErbB2 with ErbB3 in MCF7 cells expressing low/normal levels of ErbB2 (FIG. 5A) and SK-BR-3 cells expressing high levels of ErbB2 (FIG. 5B); see Example 2 below.

FIGS. 6A and 6B compare the activities of intact murine monoclonal antibody 2C4 (mu 2C4) and a chimeric 2C4 Fab fragment. FIG. 6A shows inhibition of ^{125}I-HRG binding to MCF7 cells by chimeric 2C4 Fab or intact murine monoclonal antibody 2C4. MCF7 cells were seeded in 24-well plates (1×10^5cells/well) and grown to about 85% confluency for two days. Binding experiments were conducted as described in Lewis et al. *Cancer Research* 56:1457–1465 (1996). FIG. 6B depicts inhibition of rHRGβ1 activation of p180 tyrosine phosphorylation in MCF7 cells performed as described in Lewis et al. *Cancer Research* 56:1457–1465 (1996).

FIGS. 7A and 7B depict alignments of the amino acid sequences of the variable light (V_L) (FIG. 7A) and variable heavy (V_H) (FIG. 7B) domains of murine monoclonal antibody 2C4 (SEQ ID Nos. 1 and 2, respectively); V_L and V_H domains of humanized 2C4 version 574 (SEQ ID Nos. 3 and 4, respectively), and human V_L and V_H consensus frameworks (hum κ1, light kappa subgroup I; humIII, heavy subgroup III) (SEQ ID Nos. 5 and 6, respectively). Asterisks identify differences between humanized 2C4 version 574 and murine monoclonal antibody 2C4 or between humanized 2C4 version 574 and the human framework. Complementarity Determining Regions (CDRs) are in brackets.

FIGS. 8A to C show binding of chimeric Fab 2C4 (Fab.v1) and several humanized 2C4 variants to ErbB2 extracellular domain (ECD) as determined by ELISA in Example 3.

FIG. 9 is a ribbon diagram of the V_L and V_H domains of monoclonal antibody 2C4 with white CDR backbone labeled (L1, L2, L3, H1, H2, H3). V_H sidechains evaluated by mutagenesis during humanization (see Example 3, Table 2) are also shown.

FIG. 10 depicts the effect of monoclonal antibody 2C4 or HERCEPTIN®>on EGF, TGF-α, or HRG-mediated activation of mitogen-activated protein kinase (MAPK).

FIG. 11 is a bar graph showing the effect of anti-ErbB2 antibodies (alone or in combinations) on Calu3 lung adenocarcinoma xenografts (3+ErbB2 overexpressor). Note: treatment was stopped on day 24.

FIG. 12 depicts the effect of recombinant humanized monoclonal antibody 2C4 (rhuMAb 2C4) or HERCEPTIN® on the growth of MDA-175 cells as assessed in an Alamar Blue assay.

FIG. 13 shows the efficacy of rhuMAb 2C4 against MCF7 xenografts.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. Definitions

An "ErbB receptor" is a receptor protein tyrosine kinase which belongs to the ErbB receptor family and includes EGFR, ErbB2, ErbB3 and ErbB4 receptors and other members of this family to be identified in the future. The ErbB receptor will generally comprise an extracellular domain, which may bind an ErbB ligand; a lipophilic transmembrane domain; a conserved intracellular tyrosine kinase domain; and a carboxyl-terminal signaling domain harboring several tyrosine residues which can be phosphorylated. The ErbB receptor may be a "native sequence" ErbB receptor or an "amino acid sequence variant" thereof. Preferably the ErbB receptor is native sequence human ErbB receptor.

The terms "ErbB 1", "epidermal growth factor receptor" and "EGFR" are used interchangeably herein and refer to EGFR as disclosed, for example, in Carpenter et al. *Ann. Rev. Biochem.* 56:881–914 (1987), including naturally occurring mutant forms thereof (e.g. a deletion mutant EGFR as in Humphrey et al *PNAS* (USA) 87:4207–4211 (1990)). erbB1 refers to the gene encoding the EGFR protein product.

The expressions "ErbB2" and "HER2" are used interchangeably herein and refer to human HER2 protein described, for example, in Semba et al., *PNAS (USA)* 82:6497–6501 (1985) and Yamamoto et al. *Nature* 319:230–234 (1986) (Genebank accession number X03363). The term "erbB2" refers to the gene encoding human ErbB2 and "neu" refers to the gene encoding rat $p185^{neu}$. Preferred ErbB2 is native sequence human ErbB2.

"ErbB3" and "HER3" refer to the receptor polypeptide as disclosed, for example, in U.S. Pat. Nos. 5,183,884 and 5,480,968 as well as Kraus et al. *PNAS* (USA) 86:9193–9197 (1989).

The terms "ErbB4" and "HER4" herein refer to the receptor polypeptide as disclosed, for example, in EP Pat Appln No 599,274; Plowman et al., *Proc. Natl. Acad. Sci. USA,* 90:1746–1750 (1993); and Plowman et al., *Nature,* 366:473–475 (1993), including isoforms thereof, e.g., as disclosed in WO99/19488, published Apr. 22, 1999.

By "ErbB ligand" is meant a polypeptide which binds to and/or activates an ErbB receptor. The ErbB ligand of particular interest herein is a native sequence human ErbB ligand such as epidermal growth factor (EGF) (Savage et al., *J. Biol. Chem.* 247:7612–7621 (1972)); transforming growth factor alpha (TGF-α) (Marquardt et al., *Science* 223:1079–1082 (1984)); amphiregulin also known as schwanoma or keratinocyle autocrine growth factor (Shoyab et al. *Science* 243:1074–1076(1989); Kimura et al. *Nature* 348:257–260(1990); and Cook er al. *Mol. Cell. Biol.* 11:2547–2557 (1991)); betacellulin (Shing et al., *Science* 259:1604–1607 (1993); and Sasada et al. *Biochem. Biophys. Res. Commun.* 190:1173(1993)); heparin-binding epidermal growth factor (HB-EGF)(Higashiyama et al., *Science* 251:936–939(1991)); epiregulin (Toyoda et al., *J. Biol. Chem.* 270:7495–7500(1995); and Komurasaki et al. *Oncogene* 15:2841–2848 (1997)); a heregulin (see below); neuregulin-2 (NRG-2) (Carraway et al., *Nature* 387:512–516(1997)); neuregulin-3 (NRG-3) (Zhang et al., *Proc. Natl. Acad. Sci.* 94:9562–9567(1997)); neuregulin-4 (NRG-4) (Harari et al. *Oncogene* 18:2681–89 (1999)) or cripto (CR-1) (Kannan et al. *J. Biol. Chem.* 272(6) :3330–3335 (1997)). ErbB ligands which bind EGFR include EGF, TGF-α, amphiregulin, betacellulin, HB-EGF and epiregulin. ErbB ligands which bind ErbB3 include heregulins. ErbB ligands capable of binding ErbB4 include betacellulin, epiregulin, HB-EGF, NRG-2, NRG-3, NRG-4 and heregulins.

"Heregulin" (HRG) when used herein refers to a polypeptide encoded by the heregulin gene product as disclosed in U.S. Pat. No. 5,641,869 or Marchionni et al., *Nature,* 362:312–318 (1993). Examples of heregulins include heregulin-α, heregulin-β1, heregulin-β2 and heregulin-β3 (Holmes er al., *Science,* 256:1205–1210(1992); and U.S. Pat. No. 5,641,869); neu differentiation factor (NDF) (Peles et al. *Cell* 69: 205–216 (1992)); acetylcholine receptor-inducing activity (ARIA) (Falls et al. *Cell* 72:801–815 (1993)); glial growth factors (GGFs) (Marchionni et al., *Nature,* 362:312–318(1993)); sensory and motor neuron derived factor (SMDF) (Ho et al. *J. Biol. Chem.* 270:14523–14532 (1995)); γ-heregulin (Schaefer et al. *Oncogene* 15:1385–1394 (1997)). The term includes biologically active fragments and/or amino acid sequence variants of a native sequence HRG polypeptide, such as an EGF-like domain fragment thereof (e.g. $HRG\beta1_{177-244}$).

An "ErbB hetero-oligomer" herein is a noncovalently associated oligomer comprising at least two different ErbB receptors. Such complexes may form when a cell expressing two or more ErbB receptors is exposed to an ErbB ligand and can be isolated by immunoprecipitation and analyzed by SDS-PAGE as described in Sliwkowski et al., *J. Biol. Chem.,* 269(20):14661–14665 (1994), for example. Examples of such ErbB hetero-oligomers include EGFR-ErbB2, ErbB2–ErbB3 and ErbB3–ErbB4 complexes. Moreover, the ErbB hetero-oligomer may comprise two or more ErbB2 receptors combined with a different ErbB receptor, such as ErbB3, ErbB4 or EGFR. Other proteins, such as a cytokine receptor subunit (e.g. gp130) may be included in the hetero-oligomer.

By "ligand activation of an ErbB receptor" is meant signal transduction (e.g. that caused by an intracellular kinase domain of an ErbB receptor phosphorylating tyrosine residues in the ErbB receptor or a substrate polypeptide) mediated by ErbB ligand binding to a ErbB hetero-oligomer comprising the ErbB receptor of interest. Generally, this will involve binding of an ErbB ligand to an ErbB hetero-oligomer which activates a kinase domain of one or more of the ErbB receptors in the hetero-oligomer and thereby results in phosphorylation of tyrosine residues in one or more of the ErbB receptors and/or phosphorylation of tyrosine residues in additional substrate polypeptides(s). ErbB receptor activation can be quantified using various tyrosine phosphorylation assays.

A "native sequence" polypeptide is one which has the same amino acid sequence as a polypeptide (e.g., ErbB

receptor or ErbB ligand) derived from nature. Such native sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. Thus, a native sequence polypeptide can have the amino acid sequence of naturally occurring human polypeptide, murine polypeptide, or polypeptide from any other mammalian species.

The term "amino acid sequence variant" refers to polypeptides having amino acid sequences that differ to some extent from a native sequence polypeptide. Ordinarily, amino acid sequence variants will possess at least about 70% homology with at least one receptor binding domain of a native ErbB ligand or with at least one ligand binding domain of a native ErbB receptor, and preferably, they will be at least about 80%, more preferably at least about 90% homologous with such receptor or ligand binding domains. The amino acid sequence variants possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence of the native amino acid sequence.

"Homology" is defined as the percentage of residues in the amino acid sequence variant that are identical after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology. Methods and computer programs for the alignment are well known in the art. One such computer program is "Align 2", authored by Genentech, Inc., which was filed with user documentation in the United States Copyright Office, Washington, D.C. 20559, on Dec. 10, 1991.

The term "antibody" herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two intact antibodies, and antibody fragments, so long as they exhibit the desired biological activity.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al, *Nature,* 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., *Nature,* 352:624–628 (1991) and Marks et al., *J. Mol. Biol.,* 222:581–597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al, *Proc. Natl. Acad. Sci. USA,* 81:6851–6855 (1984)). Chimeric antibodies of interest herein include "primatized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (e.g. Old World Monkey, Ape etc) and human constant region sequences.

"Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s).

An "intact" antibody is one which comprises an antigen-binding variable region as well as a light chain constant domain (C_L) and heavy chain constant domains, C_H1, C_H2 and C_H3. The constant domains may be native sequence constant domains (e.g. human native sequence constant domains) or amino acid sequence variant thereof. Preferably, the intact antibody has one or more effector functions.

Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor; BCR), etc.

Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different "classes". There are five-major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called α, δ, ϵ, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

"Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells in summarized is Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457–92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. *PNAS* (USA) 95:652–656 (1998).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred. The effector cells may be isolated from a native source thereof, e.g. from blood or PBMCs as described herein.

The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fe region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and Fcγ RIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see review M. in Daëron, *Annu. Rev. Immunol.* 15:203–234 (1997)). FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol 9:457–92 (1991); Capel et al., *Immunomethods* 4:25–34 (1994); and de Haas et al., *J. Lab. Clin. Med.* 126:330–41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.* 117:587 (1976) and Kim et al., *J. Immunol.* 24:249 (1994)).

"Complement dependent cytotoxicity" or "CDC" refers to the ability of a molecule to lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g. an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996), may be performed.

"Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end. The constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the >sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g. residues 24–34 (L1), 50–56 (L2) and 89–97 (L3) in the light chain variable domain and 31–35 (H1), 50–65 (H2) and 95–102 (H3) in the heavy chain variable domain; Kabat et al., *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop" (e.g. residues 2632 (L1), 50–52 (L2) and 91–96 (L3) in the light chain variable domain and 26–32 (H1), 53–55 (H2) and 96–101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.* 196:901–917 (1987)). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH I) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

"Single-chain Fv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-

Verlag, New York, pp. 269–315 (1994). Anti-ErbB2 antibody scFv fragments are described in WO93/16185; U.S. Pat. No. 5,571,894; and U.S. Pat. No. 5,587,458.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a variable heavy domain (V_H) connected to a variable light domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., *Proc. Natl. Acad. Sci. USA,* 90:6444–6448 (1993).

"Humanized" forms of non-human (e.g., rodent) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., *Nature* 321:522–525 (1986); Riechmann et al., *Nature* 332:323–329(1988); and Presta, *Curr. Op. Struct. Biol.* 2:593–596 (1992).

Humanized anti-ErbB2 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (HERCEPTIN®) as described in Table 3 of U.S. Pat. No. 5,821,337 expressly incorporated herein by reference; humanized 520C9 (WO93/21319) and humanized 2C4 antibodies as described hereinbelow.

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

An antibody "which binds" an antigen of interest, e.g. ErbB2 antigen, is one capable of binding that antigen with sufficient affinity such that the antibody is useful as a therapeutic agent in targeting a cell expressing the antigen. Where the antibody is one which binds ErbB2, it will usually preferentially bind ErbB2 as opposed to other ErbB receptors, and may be one which does not significantly cross-react with other proteins such as EGFR, ErbB3 or ErbB4. In such embodiments, the extent of binding of the antibody to these non-ErbB2 proteins (e.g., cell surface binding to endogenous receptor) will be less than 10% as determined by fluorescence activated cell sorting (FACS) analysis or radioimmunoprecipitation (RIA). Sometimes, the anti-ErbB2 antibody will not significantly cross-react with the rat neu protein, e.g., as described in Schecter et al. *Nature* 312:513 (1984) and Drebin et al., *Nature* 312:545–548(1984).

An antibody which "blocks" ligand activation of an ErbB receptor is one which reduces or prevents such activation as hereinabove defined, wherein the antibody is able to block ligand activation of the ErbB receptor substantially more effectively than monoclonal antibody 4D5, e.g. about as effectively as monoclonal antibodies 7F3 or 2C4 or Fab fragments thereof and preferably about as effectively as monoclonal antibody 2C4 or a Fab fragment thereof. For example, the antibody that blocks ligand activation of an ErbB receptor may be one which is about 50–100% more effective than 4D5 at blocking formation of an ErbB hetero-oligomer. Blocking of ligand activation of an ErbB receptor can occur by any means, e.g. by interfering with: ligand binding to an ErbB receptor, ErbB complex formation, tyrosine kinase activity of an ErbB receptor in an ErbB complex and/or phosphorylation of tyrosine kinase residue (s) in or by an ErbB receptor. Examples of antibodies which block ligand activation of an ErbB receptor include monoclonal antibodies 2C4 and 7F3 (which block HRG activation of ErbB2/ErbB3 and ErbB2/ErbB4 hetero-oligomers; and EGF, TGF-α, amphiregulin, HB-EGF and/or epiregulin activation of an EGFR/ErbB2 hetero-oligomer); and L26, L96 and L288 antibodies (Klapper et al. *Oncogene* 14:2099–2109(1997)), which block EGF and NDF binding to T47D cells which express EGFR, ErbB2, ErbB3 and ErbB4.

An antibody having a "biological characteristic" of a designated antibody, such as the monoclonal antibody designated 2C4, is one which possesses one or more of the biological characteristics of that antibody which distinguish it from other antibodies that bind to the same antigen (e.g. ErbB2). For example, an antibody with a biological characteristic of 2C4 may block HRG activation of an ErbB hetero-oligomer comprising ErbB2 and ErbB3 or ErbB4; block EGF, TGF-α, HB-EGF, epiregulin and/or amphiregulin activation of an ErbB receptor comprising EGFR and ErbB2; block EGF, TGF-α and/or HRG mediated activation of MAPK, and/or bind the same epitope in the extracellular domain of ErbB2 as that bound by 2C4 (e.g. which blocks binding of monoclonal antibody 2C4 to ErbB2).

Unless indicated otherwise, the expression "monoclonal antibody 2C4" refers to an antibody that has antigen binding residues of, or derived from, the murine 2C4 antibody of the Examples below. For example, the monoclonal antibody 2C4 may be murine monoclonal antibody 2C4 or a variant thereof, such as humanized antibody 2C4, possessing antigen binding amino acid residues of murine monoclonal antibody 2C4. Examples of humanized 2C4 antibodies are provided in Example 3 below. Unless indicated otherwise, the expression "rhuMAb 2C4" when used herein refers to an antibody comprising the variable light (V_L) and variable heavy (V_H) sequences of SEQ ID Nos. 3 and 4, respectively,

fused to human light and heavy IgG1 (non-A allotype) constant region sequences optionally expressed by a Chinese Hamster Ovary (CHO) cell.

Unless indicated otherwise, the term "monoclonal antibody 4D5" refers to an antibody that has antigen binding residues of, or derived from, the murine 4D5 antibody (ATCC CRL 10463). For example, the monoclonal antibody 4D5 may be murine monoclonal antibody 4D5 or a variant thereof, such as a humanized 4D5, possessing antigen binding residues of murine monoclonal antibody 4D5. Exemplary humanized 4D5 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (HERCEPTIN®) as in U.S. Pat. No. 5,821,337, with huMAb4D5-8 (HERCEPTIN®) being a preferred humanized 4D5 antibody.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially an ErbB expressing cancer cell either in vitro or in vivo. Thus, the growth inhibitory agent may be one which significantly reduces the percentage of ErbB expressing cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (W B Saunders: Philadelphia, 1995), especially p. 13.

Examples of "growth inhibitory" antibodies are those which bind to ErbB2 and inhibit the growth of cancer cells overexpressing ErbB2. Preferred growth inhibitory anti-ErbB2 antibodies inhibit growth of SK-BR-3 breast tumor cells in cell culture by greater than 20%, and preferably greater than 50% (e.g. from about 50% to about 100%) at an antibody concentration of about 0.5 to 30 μg/ml, where the growth inhibition is determined six days after exposure of the SK-BR-3 cells to the antibody (see U.S. Pat. No. 5,677,171 issued Oct. 14, 1997). The SK-BR-3 cell growth inhibition assay is described in more detail in that patent and hereinbelow. The preferred growth inhibitory antibody is monoclonal antibody 4D5, e.g., humanized 4D5.

An antibody which "induces cell death" is one which causes a viable cell to become nonviable. The cell is generally one which expresses the ErbB2 receptor, especially where the cell overexpresses the ErbB2 receptor. Preferably, the cell is a cancer cell, e.g. a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. In vitro, the cell may be a SK-BR-3, BT474, Calu 3, MDA-MB-453, MDA-MB-361 or SKOV3 cell. Cell death in vitro may be determined in the absence of complement and immune effector cells to distinguish cell death induced by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC). Thus, the assay for cell death may be performed using heat inactivated serum (i.e. in the absence of complement) and in the absence of immune effector cells. To determine whether the antibody is able to induce cell death, loss of membrane integrity as evaluated by uptake of propidium iodide (PI), trypan blue (see Moore et al. *Cytotechnology* 17:1–11 (1995)) or 7AAD can be assessed relative to untreated cells. Preferred cell death-inducing antibodies are those which induce PI uptake in the PI uptake assay in BT474 cells (see below).

An antibody which "induces apoptosis" is one which induces programmed cell, death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is usually one which overexpresses the ErbB2 receptor. Preferably the cell is a tumor cell, e.g. a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. In vitro, the cell may be a SK-BR-3, BT474, Calu 3 cell, MDA-MB-453, MDA-MB-361 or SKOV3 cell. Various methods are available for evaluating the cellular events associated with apoptosis. For example, phosphatidyl serine (PS) translocation can be measured by annexin binding; DNA fragmentation can be evaluated through DNA laddering; and nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells. Preferably, the antibody which induces apoptosis is one which results in about 2 to 50 fold, preferably about 5 to 50 fold, and most preferably about 10 to 50 fold, induction of annexin binding relative to untreated cell in an annexin binding assay using BT474 cells (see below). Sometimes the pro-apoptotic antibody will be one which further blocks ErbB ligand activation of an ErbB receptor (e.g. 7F3 antibody); i.e. the antibody shares a biological characteristic with monoclonal antibody 2C4. In other situations, the antibody is one which does not significantly block ErbB ligand activation of an ErbB receptor (e.g. 7C2). Further, the antibody may be one like 7C2 which, while inducing apoptosis, does not induce a large reduction in the percent of cells in S phase (e.g. one which only induces about 0–10% reduction in the percent of these cells relative to control).

The "epitope 2C4" is the region in the extracellular domain of ErbB2 to which the antibody 2C4 binds. In order to screen for antibodies which bind to the 2C4 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to assess whether the antibody binds to the 2C4 epitope of ErbB2 (e.g. any one or more residues in the region from about residue 22 to about residue 584 of ErbB2, inclusive; see FIGS. 1A–B).

The "epitope 4D5" is the region in the extracellular domain of ErbB2 to which the antibody 4D5 (ATCC CRL 10463) binds. This epitope is close to the transmembrane domain of ErbB2. To screen for antibodies which bind to the 4D5 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to assess whether the antibody binds to the 4D5 epitope of ErbB2 (e.g. any one or more residues in the region from about residue 529 to about residue 625, inclusive; see FIGS. 1A–B).

The "epitope 3H4" is the region in the extracellular domain of ErbB2 to which the antibody 3H4 binds. This epitope includes residues from about 541 to about 599, inclusive, in the amino acid sequence of ErbB2 extracellular domain; see FIGS. 1A–B.

The "epitope 7C2/7F3" is the region at the N terminus of the extracellular domain of ErbB2 to which the 7C2 and/or 7F3 antibodies (each deposited with the ATCC, see below)

bind. To screen for antibodies which bind to the 7C2 nF3 epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping can be performed to establish whether the antibody binds to the 7C2 nF3 epitope on ErbB2 (e.g. any one or more of residues in the region from about residue 22 to about residue 53 of ErbB2; see FIGS. 1A–B).

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. Hence, the mammal to be treated herein may have been diagnosed as having the disorder or may be predisposed or susceptible to the disorder.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human.

A "disorder" is any condition that would benefit from treatment with the anti-ErbB2 antibody. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors; leukemias and lymphoid malignancies; neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic and immunologic disorders.

The term "therapeutically effective amount" refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

An "ErbB-expressing cancer" is one comprising cells which have ErbB protein present at their cell surface. An "ErbB2-expressing cancer" is one which produces sufficient levels of ErbB2 at the surface of cells thereof, such that an anti-ErbB2 antibody can bind thereto and have a therapeutic effect with respect to the cancer.

A cancer "characterized by excessive activation" of an ErbB receptor is one in which the extent of ErbB receptor activation in cancer cells significantly exceeds the level of activation of that receptor in non-cancerous cells of the same tissue type. Such excessive activation may result from overexpression of the ErbB receptor and/or greater than normal levels of an ErbB ligand available for activating the ErbB receptor in the cancer cells. Such excessive activation may cause and/or be caused by the malignant state of a cancer cell. In some embodiments, the cancer will be subjected to a diagnostic or prognostic assay to determine whether amplification and/or overexpression of an ErbB receptor is occurring which results in such excessive activation of the ErbB receptor. Alternatively, or additionally, the cancer may be subjected to a diagnostic or prognostic assay to determine whether amplification and/or overexpression an ErbB ligand is occurring in the cancer which attributes to excessive activation of the receptor. In a subset of such cancers, excessive activation of the receptor may result from an autocrine stimulatory pathway.

In an "autocrine" stimulatory pathway, self stimulation occurs by virtue of the cancer cell producing both an ErbB ligand and its cognate ErbB receptor. For example, the cancer may express or overexpress EGFR and also express or overexpress an EGFR ligand (e.g. EGF, TGF-α, or HB-EGF). In another embodiment, the cancer may express or overexpress ErbB2 and also express or overexpress a heregulin (e.g. γ-HRG).

A cancer which "overexpresses" an ErbB receptor is one which has significantly higher levels of an ErbB receptor, such as ErbB2, at the cell surface thereof, compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. ErbB receptor overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the ErbB protein presenton the surface of a cell (e.g. via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of ErbB-encoding nucleic acid in the cell, e.g. via fluorescent in situ hybridization (FISH; see WO98/45479 published October, 1998), southern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). One may also study ErbB receptor overexpression by measuring shed antigen (e.g., ErbB extracellular domain) in a biological fluid such as serum (see, e.g., U.S. Pat. No. 4,933,294 issued Jun. 12, 1990; WO91/05264 published Apr. 18, 1991; U.S. Pat. No. 5,401,638 issued Mar. 28, 1995; and Sias et al. *J. Immunol. Methods* 132 73–80 (1990)). Aside from the above assays, various in vivo assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, e.g. a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, e.g. by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody.

Conversely, a cancer which is "not characterized by overexpression of the ErbB2 receptor" is one which, in a diagnostic assay, does not express higher than normal levels of ErbB2 receptor compared to a noncancerous cell of the same tissue type.

A cancer which "overexpresses" an ErbB ligand is one which produces significantly higher levels of that ligand compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification

or by increased transcription or translation. Overexpression of the ErbB ligand may be determined diagnostically by evaluating levels of the ligand (or nucleic acid encoding it) in the patient, e.g. in a tumor biopsy or by various diagnostic assays such as the IHC, FISH, southern blotting, PCR or in vivo assays described above.

A "hormone independent" cancer is one in which proliferation thereof is not dependent on the presence of a hormone which binds to a receptor expressed by cells in the cancer. Such cancers do not undergo clinical regression upon administration of pharmacological or surgical strategies that reduce the hormone concentration in or near the tumor. Examples of hormone independent cancers include androgen independent prostate cancer, estrogen independent breast cancer, endometrial cancer and ovarian cancer. Such cancers may begin as hormone dependent tumors and progress from a hormone-sensitive stage to a hormone-refractory tumor following anti-hormonal therapy.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g. At^{211}, I^{131}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carnomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxanes, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (TAXOTERE®, Rhône-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

As used herein, the term "EGFR-targeted drug" refers to a therapeutic agent that binds to EGFR and, optionally, inhibits EGFR activation. Examples of such agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, U.S. Pat. No. 4,943,533, Mendelsohn et al.) and variants thereof, such as chimerized 225 (C225) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.); antibodies that bind type II mutant EGFR (U.S. Pat. No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in U.S. Pat. No. 5,891,996; and human antibodies that bind EGFR (see WO98/50433, Abgenix). The anti-EGFR antibody may be conjugated with a cyotoxic agent, thus generating an immunoconjugate (see, e.g., EP659,439A2, Merck Patent GmbH). Examples of small molecules that bind to EGFR include ZD1839 (Astra Zeneca), CP-358774 (OSI/Pfizer) and AG1478.

An "anti-angiogenic agent" refers to a compound which blocks, or interferes with to some degree, the development of blood vessels. The anti-angiogenic factor may, for instance, be a small molecule or antibody that binds to a growth factor or growth factor receptor involved in promoting angiogenesis. The preferred anti-angiogenic factor herein is an antibody that binds to Vascular Endothelial Growth Factor (VEGF).

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse

gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-αand TGF-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon-α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

The term "prodrug" as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, e.g., Wilman, "Prodrugs in Cancer Chemotherapy" *Biochemical Society Transactions,* 14, pp. 375–382, 615th Meeting Belfast (1986) and Stella et al., "Prodrugs: A Chemical Approach to Targeted Drug Delivery," Directed Drug Delivery, Borchardt et al., (ed.), pp. 247–267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β-lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as the anti-ErbB2 antibodies disclosed herein and, optionally, a chemotherapeutic agent) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

A "cardioprotectant" is a compound or composition which prevents or reduces myocardial dysfunction (i.e. cardiomyopathy and/or congestive heart failure) associated with administration of a drug, such as an anthracycline antibiotic and/or an anti-ErbB2 antibody, to a patient. The cardioprotectant may, for example, block or reduce a free-radical-mediated cardiotoxic effect and/or prevent or reduce oxidative-stress injury. Examples of cardioprotectants encompassed by the present definition include the iron-chelating agent dexrazoxane (ICRF-187) (Seifert et al. *The Annals of Pharmacotherapy* 28:1063–1072 (1994)); a lipid-lowering agent and/or anti-oxidant such as probucol (Singal et al. *J. Mol. Cell Cardiol.* 27:1055–1063 (1995)); amifostine (aminothiol 2-[(3-aminopropyl)amino]ethanethiol-dihydrogen phosphate ester, also called WR-2721, and the dephosphorylated cellular uptake form thereof called WR-1065) and S-3-(3-methylaminopropylamino) propylphosphorothioic acid (WR-151327), see Green et al. *Cancer Research* 54:738–741 (1994); digoxin (Bristow, M. R. In: Bristow MR, ed. Drug-Induced Heart Disease. New York: Elsevier 191–215 (1980)); beta-blockers such as metoprolol (Hjalmarson et al. *Drugs* 47: Suppl 4:31–9 (1994); and Shaddy et al. *Am. Heart J.* 129:197–9(1995)); vitamin E; ascorbic acid (vitamin C); free radical scavengers such as oleanolic acid, ursolic acid and N-acetylcysteine (NAC); spin trapping compounds such as alpha-phenyl-tert-butyl nitrone (PBN); (Paracchini et al., *Anticancer Res.* 13:1607–1612 (1993)); selenoorganic compounds such as P251 (Elbesen); and the like.

An "isolated" nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the antibody nucleic acid. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the antibody where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The expression "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

II. Production of Anti-ErbB2 Antibodies

A description follows as to exemplary techniques for the production of the antibodies used in accordance with the present invention, The ErbB2 antigen to be used for production of antibodies may be, e.g., a soluble form of the extracellular domain of ErbB2 or a portion thereof, containing the desired epitope. Alternatively, cells expressing ErbB2 at their cell surface (e.g. NIH-3T3 cells transformed

to overexpress ErbB2; or a carcinoma cell line such as SK-BR-3 cells, see Stancovski et al. *PNAS* (USA) 88:8691–8695 (1991)) can be used to generate antibodies. Other forms of ErbB2 useful for generating antibodies will be apparent to those skilled in the art.

(i) Polyclonal Antibodies

Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N{=}C{=}NR$, where R and R are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with ⅕ to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) Monoclonal Antibodies

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., *Nature,* 256:495 (1975), or may be made by recombinant DNA methods (U.S. Pat. No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59–103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Md. USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.,* 133:3001(1984); and Brodeur et al. *Monoclonal Antibody Production Techniques and Applications*, pp. 51–63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson et al., *Anal. Biochem.,* 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59–103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., *Curr. Opinion in Immunol.,* 5:256–262 (1993) and Plückthun, *Immunol. Revs.,* 130:151–188 (1992).

In a further embodiment, monoclonal antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty et al., *Nature,* 348:552–554 (1990). Clackson et al. *Nature,* 352:624–628 (1991) and Marks et al., *J. Mol. Biol.,* 222:581–597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks et al., *Bio/Technology,* 10:779–783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse et al., *Nuc. Acids. Res.,* 21:2265–2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

The DNA also may be modified, for example, by substituting the coding sequence for human heavy chain and light chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567; and Morrison, et al., *Proc. Natl. Acad. Sci. USA,* 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

(iii) Humanized Antibodies

Methods for humanizing non-human antibodies have been described in the art. Preferably, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature,* 321:522–525 (1986); Riechmann et al., *Nature,* 332:323–327 (1988); Verhoeyen et al., *Science,* 239:1534–1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework region (FR) for the humanized antibody (Sims et al., *J. Immunol.,* 151:2296 (1993); Chothia et al., *J. Mol. Biol.,* 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., *Proc. Natl. Acad. Sci. USA,* 89:4285 (1992); Presta et al., *J. Immunol.,* 151:2623(1993)).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

Example 3 below describes production of exemplary humanized anti-ErbB2 antibodies which bind ErbB2 and block ligand activation of an ErbB receptor. The humanized antibody of particular interest herein blocks EGF, TGF-α and/or HRG mediated activation of MAPK essentially as effectively as murine monoclonal antibody 2C4 (or a Fab fragment thereof) and/or binds ErbB2 essentially as effectively as murine monoclonal antibody 2C4 (or a Fab fragment thereof). The humanized antibody herein may, for example, comprise nonhuman hypervariable region residues incorporated into a human variable heavy domain and may further comprise a framework region (FR) substitution at a position selected from the group consisting of 69H, 71H and 73H utilizing the variable domain numbering system set forth in Kabat et al., *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). In one embodiment, the humanized antibody comprises FR substitutions at two or all of positions 69H, 71H and 73H.

An exemplary humanized antibody of interest herein comprises variable heavy domain complementarity determining residues GFTFTDYTMX, where X is preferrably D or S (SEQ ID NO:7); DVNPNSGGSIYNQRFKG (SEQ ID NO:8); and/or NLGPSFYFDY (SEQ ID NO:9), optionally comprising amino acid modifications of those CDR residues, e.g. where the modifications essentially maintain or improve affinity of the antibody. For example, the antibody variant of interest may have from about one to about seven or about five amino acid substitutions in the above variable heavy CDR sequences. Such antibody variants may be prepared by affinity maturation, e.g., as described below. The most preferred humanized antibody comprises the variable heavy domain amino acid sequence in SEQ ID NO:4.

The humanized antibody may comprise variable light domain complementarity determining residues KASQDVSIGVA (SEQ ID NO:10); $SASYX^1X^2X^3$, where X^1 is preferably R or L, X^2 is preferably Y or E, and X^3 is preferably T or S (SEQ ID NO:11); and/or QQYYIYPYT (SEQ ID NO:12), e.g. in addition to those variable heavy domain CDR residues in the preceding paragraph. Such humanized antibodies optionally comprise amino acid modifications of the above CDR residues, e.g. where the modifications essentially maintain or improve affinity of the antibody. For example, the antibody variant of interest may have from about one to about seven or about five amino acid substitutions in the above variable light CDR sequences. Such antibody variants may be prepared by affinity maturation, e.g., as described below. The most preferred humanized antibody comprises the variable light domain amino acid sequence in SEQ ID NO:3.

The present application also contemplates affinity matured antibodies which bind ErbB2 and block ligand activation of an ErbB receptor. The parent antibody may be a human antibody or a humanized antibody, e.g., one comprising the variable light and/or heavy sequences of SEQ ID Nos. 3 and 4, respectively (i.e. variant 574). The affinity matured antibody preferably binds to ErbB2 receptor with an affinity superior to that of murine 2C4 or variant 574 (e.g. from about two or about four fold, to about 100 fold or about 1000 fold improved affinity, e.g. as assessed using a ErbB2-

extracellular domain (ECD) ELISA). Exemplary variable heavy CDR residues for substitution include H28, H30, H34, H35, H64, H96, H99, or combinations of two or more (e.g. two, three, four, five, six, or seven of these residues). Examples of variable light CDR residues for alteration include L28, L50, L53, L56, L91, L92, L93, L94, L96, L97 or combinations of two or more (e.g. two to three, four, five or up to about ten of these residues).

Various forms of the humanized antibody or affinity matured antibody are contemplated. For example, the humanized antibody or affinity matured antibody may be an antibody fragment, such as a Fab, which is optionally conjugated with one or more cytotoxic agent(s) in order to generate an immunoconjugate. Alternatively, the humanized antibody or affinity matured antibody may be an intact antibody, such as an intact IgG1 antibody.

(iv) Human Antibodies

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., *Proc. Natl. Acad. Sci. USA,* 90:2551 (1993); Jakobovits et al., *Nature,* 362:255–258 (1993); Bruggermann et al., *Year in Immuno.,* 7:33 (1993); and U.S. Pat. Nos. 5,591,669, 5,589,369 and 5,545,807.

Alternatively, phage display technology (McCafferty et al., *Nature* 348:552–553 (1990)) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g., Johnson, Kevin S. and Chiswell, David J., Current Opinion in Structural Biology 3:564–571(1993). Several sources of V-gene segments can be used for phage display. Clackson et al., *Nature,* 352:624–628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., *J. Mol. Biol.* 222:581–597 (1991), or Griffith et al., *EMBO J.* 12:725–734 (1993). See, also, U.S. Pat. Nos. 5,565,332 and 5,573,905.

As discussed above, human antibodies may also be generated by in vitro activated B cells (see U.S. Pat. Nos. 5,567,610 and 5,229,275).

Human anti-ErbB2 antibodies are described in U.S. Pat. No. 5,772,997 issued Jun. 30, 1998 and WO 97/00271 published Jan. 3, 1997.

(v) Antibody Fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., *Journal of Biochemical and Biophysical Methods* 24:107–117(1992); and Brennan et al., *Science,* 229:81(1985)). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab)_2$ fragments (Carter et al., *Bio/Technology* 10: 163–167 (1992)). According to another approach, $F(ab)_2$ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO93/16185; U.S. Pat. No. 5,571,894; and U.S. Pat. No. 5,587,458. The antibody fragment may also be a "linear antibody", e.g., as described in U.S. Pat. No. 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

(vi) Bispecific Antibodies

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the ErbB2 protein. Other such antibodies may combine an ErbB2 binding site with binding site(s) for EGFR, ErbB3 and/or ErbB4. Alternatively, an anti-ErbB2 arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2 or CD3), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the ErbB2-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express ErbB2. These antibodies possess an ErbB2-binding arm and an arm which binds the cytotoxic agent (e.g. saporin, anti-interferon-α, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. $F(ab')_2$ bispecific antibodies).

WO 96/16673 describes a bispecific anti-ErbB2/anti-FcγRIII antibody and U.S. Pat. No. 5,837,234 discloses a bispecific anti-ErbB2/anti-FcγRI antibody. A bispecific anti-ErbB2/Fcα antibody is shown in WO98/02463. U.S. Pat. No. 5,821,337 teaches a bispecific anti-ErbB2/anti-CD3 antibody.

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein et al., *Nature,* 305:537–539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker et al., *EMBO J.,* 10:3655–3659 (1991).

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an

immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology,* 121:210 (1986).

According to another approach described in U.S. Pat. No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., *Science,* 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab)_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immunobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.,* 175: 217–225 (1992) describe the production of a fully humanized bispecific antibody $F(ab)_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., *J. Immunol.,* 148(5):1547–1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., *Proc. Natl. Acad. Sci. USA,* 90:6444–6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., *J. Immunol.,* 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al. *J. Immunol.* 147:60 (1991).

(vii) Other Amino Acid Sequence Modifications

Amino acid sequence modification(s) of the anti-ErbB2 antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the anti-ErbB2 antibody are prepared by introducing appropriate nucleotide changes into the anti-ErbB2 antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the anti-ErbB2 antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the anti-ErbB2 antibody, such as changing the number or position of glycosylation sites.

A useful method for identification of certain residues or regions of the anti-ErbB2 antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells Science, 244:1081–1085 (1989). Here, a residue or group of target residues are identified (e.g., charged residues such as arg,

asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with ErbB2 antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed anti-ErbB2 antibody variants are screened for the desired activity.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an anti-ErbB2 antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the anti-ErbB2 antibody molecule include the fusion to the N- or C-terminus of the anti-ErbB2 antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the anti-ErbB2 antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

TABLE 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gin, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

Any cysteine residue not involved in maintaining the proper conformation of the anti-ErbB2 antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g. 6–7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g. binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and human ErbB2. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The

alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

Nucleic acid molecules encoding amino acid sequence variants of the anti-ErbB 2 anti body are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the anti-ErbB2 antibody.

It may be desirable to modify the antibody of the invention with respect to effector function, e.g. so as to enhance antigen-dependent cell-mediated cyotoxicity (ADCC) and/or complement dependent cytoloxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., *J. Exp Med.* 176:1191–1195 (1992) and Shopes, B. *J. Immunol.* 148:2918–2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff et al. *Cancer Research* 53:2560–2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al. *Anti-Cancer Drug Design* 3:219–230 (1989).

To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Pat. No. 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the in vivo serum half-life of the IgG molecule.

(viii) Screening for Antibodies with the Desired Properties

Techniques for generating antibodies have been described above. One may further select antibodies with certain biological characteristics, as desired.

To identify an antibody which blocks ligand activation of an ErbB receptor, the ability of the antibody to block ErbB ligand binding to cells expressing the ErbB receptor (e.g. in conjugation with another ErbB receptor with which the ErbB receptor of interest forms an ErbB hetero-oligomer) may be determined. For example, cells naturally expressing, or transfected to express, ErbB receptors of the ErbB hetero-oligomer may be incubated with the antibody and then exposed to labeled ErbB ligand. The ability of the anti-ErbB2 antibody to block ligand binding to the ErbB receptor in the ErbB hetero-oligomer may then be evaluated.

For example, inhibition of HRG binding to MCF7 breast tumor cell lines by anti-ErbB2 antibodies may be performed using monolayer MCF7 cultures on ice in a 24-well-plate format essentially as described in Example 1 below. Anti-ErbB2 monoclonal antibodies may be added to each well and incubated for 30 minutes. ^{125}I-labeled rHRG$\beta 1_{177\text{-}224}$ (25 pm) may then be added, and the incubation may be continued for 4 to 16 hours. Dose response curves may be prepared and an IC_{50} value may be calculated for the antibody of interest. In one embodiment, the antibody which blocks ligand activation of an ErbB receptor will have an IC_{50} for inhibiting HRG binding to MCF7 cells in this assay of about 50 nM or less, more preferably 10 nM or less. Where the antibody is an antibody fragment such as a Fab fragment, the IC_{50} for inhibiting HRG binding to MCF7 cells in this assay may, for example, be about 100 nM or less, more preferably 50 nM or less.

Alternatively, or additionally, the ability of the anti-ErbB2 antibody to block ErbB ligand-stimulated tyrosine phosphorylation of an ErbB receptor present in an ErbB hetero-oligomer may be assessed. For example, cells endogenously expressing the ErbB receptors or transfected to expressed them may be incubated with the antibody and then assayed for ErbB ligand-dependent tyrosine phosphorylation activity using an anti-phospholyrosine monoclonal (which is optionally conjugated with a detectable label). The kinase receptor activation assay described in U.S. Pat. No. 5,766,863 is also available for determining ErbB receptor activation and blocking of that activity by an antibody.

In one embodiment, one may screen for an antibody which inhibits HRG stimulation of p180 tyrosine phosphorylation in MCF7 cells essentially as described in Example 1 below. For example, the MCF7 cells may be plated in 24-well plates and monoclonal antibodies to ErbB2 may be added to each well and incubated for 30 minutes at room temperature; then rHRG$\beta 1_{177\text{-}244}$ may be added to each well to a final concentration of 0.2 nM, and the incubation may be continued for 8 minutes. Media may be aspirated from each well, and reactions may be stopped by the addition of 100 μl of SDS sample buffer (5% SDS, 25 mM DTT, and 25 mM Tris-HCl, pH 6.8). Each sample (25 μl) may be electrophoresed on a 4–12% gradient gel (Novex) and then electrophoretically transferred to polyvinylidene difluoride membrane. Antiphosphotyrosine (at 1 μg/ml) immunoblots may be developed, and the intensity of the predominant reactive band at M_r-180,000 may be quantified by reflectance densitometry. The antibody selected will preferably significantly inhibit HRG stimulation of p180 tyrosine phosphorylation to about 0–35% of control in this assay. A dose-response curve for inhibition of HRG stimulation of p180 tyrosine phosphorylation as determined by reflectance densitometry may be prepared and an IC_{50} for the antibody of interest may be calculated. In one embodiment, the antibody which blocks ligand activation of an ErbB receptor will have an IC_{50} for inhibiting HRG stimulation of p 180 tyrosine phosphorylation in this assay of about 50 nM or less, more preferably 10 nM or less. Where the antibody is an antibody fragment such as a Fab fragment, the IC_{50} for inhibiting HRG stimulation of p180 tyrosine phosphorylation in this assay may, for example, be about 100 nM or less, more preferably 50 nM or less.

One may also assess the growth inhibitory effects of the antibody on MDA-MB-175 cells, e.g, essentially as described in Schaefer et al. *Oncogene* 15:1385–1394 (1997). According to this assay, MDA-MB-175 cells may treated with an anti-ErbB2 monoclonal antibody (10 μg/mL) for 4 days and stained with crystal violet. Incubation with an anti-ErbB2 antibody may show a growth inhibitory effect on this cell line similar to that displayed by monoclonal antibody 2C4. In a further embodiment, exogenous HRG will not significantly reverse this inhibition. Preferably, the antibody will be able to inhibit cell proliferation of MDA-MB-175 cells to a greater extent than monoclonal antibody 4D5 (and optionally to a greater extent than monoclonal antibody 7F3), both in the presence and absence of exogenous HRG.

In one embodiment, the anti-ErbB2 antibody of interest may block heregulin dependent association of ErbB2 with

ErbB3 in both MCF7 and SK-BR-3 cells as determined in a co-immunoprecipitation experiment such as that described in Example 2 substantially more effectively than monoclonal antibody 4D5, and preferably substantially more effectively than monoclonal antibody 7F3.

To identify growth inhibitory anti-ErbB2 antibodies, one may screen for antibodies which inhibit the growth of cancer cells which overexpress ErbB2. In one embodiment, the growth inhibitory antibody of choice is able to inhibit growth of SK-BR-3 cells in cell culture by about 20–100% and preferably by about 50–100% at an antibody concentration of about 0.5 to 30 μg/ml. To identify such antibodies, the SK-BR-3 assay described in U.S. Pat. No. 5,677,171 can be performed. According to this assay, SK-BR-3 cells are grown in a 1:1 mixture of F12 and DMEM medium supplemented with 10% fetal bovine serum, glutamine and penicillin streptomycin. The SK-BR-3 cells are plated at 20,000 cells in a 35 mm cell culture dish (2 mls/35 mm dish). 0.5 to 30 μg/ml of the anti-ErbB2 antibody is added per dish. After six days, the number of cells, compared to untreated cells are counted using an electronic COULTER™ cell counter. Those antibodies which inhibit growth of the SK-BR-3 cells by about 20–100% or about 50–100% may be selected as growth inhibitory antibodies.

To select for antibodies which induce cell death, loss of membrane integrity as indicated by, e.g., PI, trypan blue or 7AAD uptake may be assessed relative to control. The preferred assay is the PI uptake assay using BT474 cells. According to this assay, BT474 cells (which can be obtained from the American Type Culture Collection (Rockville, Md.)) are cultured in Dulbecco's Modified Eagle Medium (D-MEM): Ham's F-12 (50:50) supplemented with 10% heat-inactivated FBS (Hyclone) and 2 mM L-glutamine. (Thus, the assay is performed in the absence of complement and immune effector cells). The BT474 cells are seeded at a density of 3×10^6 per dish in 100×20 mm dishes and allowed to attach overnight. The medium is then removed and replaced with fresh medium alone or medium containing 10 μg/ml of the appropriate monoclonal antibody. The cells are incubated for a 3 day time period. Following each treatment, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged at 1200 rpm for 5 minutes at 4° C., the pellet resuspended in 3 ml ice cold Ca^{2+} binding buffer (10 mM Hepes, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) and aliquoted into 35 mm strainer-capped 12×75 tubes (1 ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10 μg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those antibodies which induce statistically significant levels of cell death as determined by PI uptake may be selected as cell death-inducing antibodies.

In order to select for antibodies which induce apoptosis, an annexin binding assay using BT474 cells is available. The BT474 cells are cultured and seeded in dishes as discussed in the preceding paragraph. The medium is then removed and replaced with fresh medium alone or medium containing 10 μg/ml of the monoclonal antibody. Following a three day incubation period, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged, resuspended in Ca binding buffer and aliquoted into tubes as discussed above for the cell death assay. Tubes then receive labeled annexin (e.g. annexin V-FITC) (1 μg/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those antibodies which induce statistically significant levels of annexin binding relative to control are selected as apoptosis-inducing antibodies.

In addition to the annexin binding assay, a DNA staining assay using BT474 cells is available. In order to perform this assay, BT474 cells which have been treated with the antibody of interest as described in the preceding two paragraphs are incubated with 9 μg/ml HOECHST 33342™ for 2 hr at 37° C., then analyzed on an EPICS ELITE™ flow cytometer (Coulter Corporation) using MODFIT LT™ software (Verity Software House). Antibodies which induce a change in the percentage of apoptotic cells which is 2 fold or greater (and preferably 3 fold or greater) than untreated cells (up to 100% apoptotic cells) may be selected as pro-apoptotic antibodies using this assay.

To screen for antibodies which bind to an epitope on ErbB2 bound by an antibody of interest, a routine cross-blocking assay such as that described in Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, or additionally, epitope mapping can be performed by methods known in the art (see, e.g. FIGS. 1A and 1B herein).

(ix) Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g. a small molecule toxin or an enzymatically active toxin of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof), or a radioactive isotope (ie., a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, a maytansine (U.S. Pat. No. 5,208,020), a trichothene, and CC 1065 are also contemplated herein.

In one preferred embodiment of the invention, the antibody is conjugated to one or more maytansine molecules (e.g. about 1 to about 10 maytansine molecules per antibody molecule). Maytansine may, for example, be converted to May-SS-Me which may be reduced to May-SH3 and reacted with modified antibody (Chari et al *Cancer Research* 52: 127–131 (1992)) to generate a maytansinoid-antibody immunoconjugate.

Another immunoconjugate of interest comprises an anti-ErbB2 antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. Structural analogues of calicheamicin which may be used include, but are not limited to, γ_1^1, α_2^1, α_3^1, N-acetyl-γ_1^1, PSAG and θ_1^1, (Hinman et al. *Cancer Research* 53: 3336–3342 (1993) and Lode et al. *Cancer Research* 58: 2925–2928 (1998)). See, also, U.S. Pat. Nos. 5,714,586; 5,712,374; 5,264,586; and 5,773,001 expressly incorporated herein by reference.

Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published Oct. 28, 1993.

The present invention further contemplates an immunoconjugate formed between an antibody and a compound with nucleolytic activity (e.g. a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

A variety of radioactive isotopes are available for the production of radioconjugated anti-ErbB2 antibodies.

Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu.

Conjugates of the antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol)propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis(p-azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al. *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, dimethyl linker or disulfide-containing linker (Chari et al. *Cancer Research* 52: 127–131 (1992)) may be used.

Alternatively, a fusion protein comprising the anti-ErbB2 antibody and cytotoxic agent may be made, e.g. by recombinant techniques or peptide synthesis.

In yet another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide).

(x) Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT)

The antibodies of the present invention may also be used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (e.g. a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Pat. No. 4,975,278.

The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to covert it into its more active, cytotoxic form.

Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs, β-lactamase useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin Vamidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, e.g., Massey, Nature 328: 457–458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

The enzymes of this invention can be covalently bound to the anti-ErbB2 antibodies by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, e.g., Neuberger et al., *Nature,* 312: 604–608 (1984).

(xi) Other Antibody Modifications

Other modifications of the antibody are contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The antibody also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., (1980).

The anti-ErbB2 antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., *Proc. Natl. Acad. Sci. USA,* 82:3688 (1985); Hwang et al., *Proc. Natl. Acad. Sci. USA,* 77:4030 (1980); U.S. Pat. Nos. 4,485,045 and 4,544,545; and WO97/38731 published Oct. 23, 1997. Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin et al. *J. Biol. Chem.* 257: 286–288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon et al. *J. National Cancer Inst.* 81(19)1484 (1989).

III. Vectors, Host Cells and Recombinant Methods

The invention also provides isolated nucleic acid encoding the humanized anti-ErbB2 antibody, vectors and host cells comprising the nucleic acid, and recombinant techniques for the production of the antibody.

For recombinant production of the antibody, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the monoclonal antibody is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of

replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

(i) Signal Sequence Component

The anti-ErbB2 antibody of this invention may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected preferably is one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native anti-ErbB2 antibody signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, 1 pp, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, e.g. the yeast invertase leader, a factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders), or acid phosphatase leader, the *C. albicans* glucoamylase leader, or the signal described in WO 90/13646. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available.

The DNA for such precursor region is ligated in reading frame to DNA encoding the anti-ErbB2 antibody.

(ii) Origin of Replication Component

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

(iii) Selection Gene Component

Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for Bacilli.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the anti-ErbB2 antibody nucleic acid, such as DHFR, thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc.

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity.

Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding anti-ErbB2 antibody, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Pat. No. 4,965,199.

A suitable selection gene for use in yeast is the trp1 gene present in the yeast plasmid YRp7 (Stinchcomb et al, *Nature,* 282:39 (1979)). The trp1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, Genetics, 85:12 (1977). The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, Leu2-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the Leu2 gene.

In addition, vectors derived from the 1.6 μm circular plasmid pKD1 can be used for transformation of *Kluyveromyces* yeasts. Alternatively, an expression system for large-scale production of recombinant calf chymosin was reported for *K. lactis* Van den Berg, Bio/technology, 8:135 (1990). Stable multi-copy expression vectors for secretion of mature recombinant human serum albumin by industrial strains of *Kluyveromyces* have also been disclosed. Fleer et al, *Bio/Technology,* 9:968–975 (1991).

(iv) Promoter Component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the anti-ErbB2 antibody nucleic acid. Promoters suitable for use with prokaryotic hosts include the phoA promoter, β-lactamase and lactose promoter systems, alkaline phosphatase, a tryptophan (trp) promoter system, and hybrid promoters such as the tac promoter. However, other known bacterial promoters are suitable. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the anti-ErbB2 antibody.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use

in yeast expression are further described in EP 73,657. Yeast enhancers also are advantageously used with yeast promoters.

Anti-ErbB2 antibody transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papillomavirus as a vector is disclosed in U.S. Pat. No. 4,419,446. A modification of this system is described in U.S. Pat. No. 4,601,978. See also Reyes et al., *Nature* 297:598–601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the rous sarcoma virus long terminal repeat can be used as the promoter.

(v) Enhancer Element Component

Transcription of a DNA encoding the anti-ErbB2 antibody of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100–270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature* 297:17–18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the anti-ErbB2 antibody-encoding sequence, but is preferably located at a site 5' from the promoter.

(vi) Transcription Termination Component

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3, untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding anti-ErbB2 antibody. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

(vii) Selection and Transformation of Host Cells.

Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include *eubacteria*, such as Gram-negative or Grain-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g., *E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella*, e.g., *Salmonella typhimurium, Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 Apr. 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X11776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for anti-ErbB2 antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe; Kluyveromyces* hosts such as, e.g., *K. lactis, K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045). *K. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilarum* (ATCC 36,906), *K. thermotolerans*, and *K. marxianus; yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida; Trichoderma reesia* (EP 244,234); *Neurospora crassa; Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, e.g., *Neurospora, Penicillium, Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger.*

Suitable host cells for the expression of glycosylated anti-ErbB2 antibody are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can also be utilized as hosts.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR(CHO, Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243–251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TR1 cells (Mather et al. *Annals N.Y. Acad. Sci.* 383:44–68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors for anti-ErbB2 antibody production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

(viii) Culturing the Host Cells

The host cells used to produce the anti-ErbB2 antibody of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., *Meth. Enz.* 58:44 (1979), Barnes et al., *Anal. Biochem.* 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Pat. No. Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

(ix) Purification of Anti-ErbB2 Antibody

When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Carter et al., *Bio/Technology* 10:163–167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2, or γ4 heavy chains (Lindmark et al., *J. Immunol. Meth.* 62:1–13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss et al., *EMBO J.* 5:15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a C_H3 domain, the Bakerbond ABX™resin (J. T. Baker, Phillipsburg, N.J.) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSE™ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.54.5, preferably performed at low salt concentrations (e.g., from about 0–0.25M salt).

IV. Pharmaceutical Formulations

Therapeutic formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG). Preferred lyophilized anti-ErbB2 antibody formulations are described in WO 97/04801, expressly incorporated herein by reference.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide antibodies which bind to EGFR, ErbB2 (e.g. an antibody which binds a different epitope on ErbB2), ErbB3, ErbB4, or vascular endothelial factor (VEGF) in the one formulation. Alternatively, or additionally, the composition may further comprise a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, EGFR-targeted drug, anti-angiogenic agent, and/or cardioprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and y ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(−)-3-hydroxybutyric acid.

The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

V. Treatment with the Anti-ErbB2 Antibodies

It is contemplated that, according to the present invention, the anti-ErbB2 antibodies may be used to treat various diseases or disorders. Exemplary conditions or disorders include benign or malignant tumors; leukemias and lymphoid malignancies; other disorders such as neuronal, glial, astrocytal, hypothalamic, glandular, macrophagal, epithelial, stromal, blastocoelic, inflammatory, angiogenic and immunologic disorders.

Generally, the disease or disorder to be treated is cancer. Examples of cancer to be treated herein include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

The cancer will general ly comprise ErbB2-expressing cells, such that the anti-ErbB2 antibody herein is able to bind to the cancer. While the cancer may be characterized by overexpression of the ErbB2 receptor, the present application further provides a method for treating cancer which is not considered to be an ErbB2-overexpressing cancer. To determine ErbB2 expression in the cancer, various diagnostic/prognostic assays are available. In one embodiment, ErbB2 overexpression may be analyzed by IHC, e.g. using the HERCEPTEST® (Dako). Parrafin embedded tissue sections from a tumor biopsy may be subjected to the IHC assay and accorded a ErbB2 protein staining intensity criteria as follows:

Score 0

no staining is observed or membrane staining is observed in less than 10% of tumor cells.

Score 1+

a faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.

Score 2+

a weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

Score 3+

a moderate to strong complete membrane staining is observed in more than 10% of the tumor cells.

Those tumors with 0 or 1+scores for ErbB2 overexpression assessment may be characterized as not overexpressing ErbB2, whereas those tumors with 2+ or 3+scores may be characterized as overexpressing ErbB2.

Alternatively, or additionally, FISH assays such as the INFORM™ (sold by Ventana, Arizona) or PATHVISION™ (Vysis, Ill.) may be carried out on formalin-fixed, paraffin-embedded tumor tissue to determine the extent (if any) of ErbB2 overexpression in the tumor.

In one embodiment, the cancer will be one which expresses (and may overexpress) EGFR. Examples of cancers which may express/overexpress EGFR include squamous cell cancer (e.g. epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

The cancer to be treated herein may be one characterized by excessive activation of an ErbB receptor, e.g. EGFR. Such excessive activation may be attributable to overexpression or increased production of the ErbB receptor or an ErbB ligand. In one embodiment of the invention, a diagnostic or prognostic assay will be performed to determine whether the patient's cancer is characterized by excessive activation of an ErbB receptor. For example, ErbB gene amplification and/or overexpression of an ErbB receptor in the cancer may be determined. Various assays for determining such amplification/overexpression are available in the art and include the IHC, FISH and shed antigen assays described above. Alternatively, or additionally, levels of an ErbB ligand, such as TGF-α, in or associated with the tumor may be determined according to known procedures. Such assays may detect protein and/or nucleic acid encoding it in the sample to be tested. In one embodiment, ErbB ligand levels in the tumor may be determined using immunohistochemistry (IHC); see, for example, Scher et al. *Clin. Cancer Research* 1:545–550 (1995). Alternatively, or additionally, one may evaluate levels of ErbB ligand-encoding nucleic acid in the sample to be tested; e.g. via FISH, southern blotting, or PCR techniques.

Moreover, ErbB receptor or ErbB ligand overexpression or amplification may be evaluated using an in vivo diagnostic assay, e.g. by administering a molecule (such as an antibody) which binds the molecule to be detected and is tagged with a detectable label (e.g. a radioactive isotope) and externally scanning the patient for localization of the label.

Where the cancer to be treated is hormone independent cancer, expression of the hormone (e.g. androgen) and/or its cognate receptor in the tumor may be assessed using any of the various assays available, e.g. as described above. Alternatively, or additionally, the patient may be diagnosed as having hormone independent cancer in that they no longer respond to anti-androgen therapy.

In certain embodiments, an immunoconjugate comprising the anti-ErbB2 antibody conjugated with a cytotoxic agent is administered to the patient. Preferably, the immunoconju-

gate and/or ErbB2 protein to which it is bound is/are internalized by the cell, resulting in increased therapeutic efficacy of the immunoconjugate in killing the cancer cell to which it binds. In a preferred embodiment, the cytotoxic agent targets or interferes with nucleic acid in the cancer cell. Examples of such cytotoxic agents include maytansinoids, calicheamicins, ribonucleases and DNA endonucleases.

The anti-ErbB2 antibodies or immunoconjugates are administered to a human patient in accord with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous or subcutaneous administration of the antibody is preferred.

Other therapeutic regimens may be combined with the administration of the anti-ErbB2 antibody. The combined administration includes coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

In one preferred embodiment, the patient is treated with two different anti-ErbB2 antibodies. For example, the patient may be treated with a first anti-ErbB2 antibody which blocks ligand activation of an ErbB receptor or an antibody having a biological characteristic of monoclonal antibody 2C4 as well as a second anti-ErbB2 antibody which is growth inhibitory (e.g. HERCEPTIN®) or an anti-ErbB2 antibody which induces apoptosis of an ErbB2-overexpressing cell (e.g. 7C2, 7F3 or humanized variants thereof). Preferably such combined therapy results in a synergistic therapeutic effect. One may, for instance, treat the patient with HERCEPTIN® and thereafter treat with rhuMAb 2C4, e.g. where the patient does not respond to HERCEPTIN® therapy. In another embodiment, the patient may first be treated with rhuMAb 2C4 and then receive HERCEPTIN® therapy. In yet a further embodiment, the patient may be treated with both rhuMAb 2C4 and HERCEPTIN® simultaneously.

It may also be desirable to combine administration of the anti-ErbB2 antibody or antibodies, with administration of an antibody directed against another tumor associated antigen. The other antibody in this case may, for example, bind to EGFR, ErbB3, ErbB4, or vascular endothelial growth factor (VEGF).

In one embodiment, the treatment of the present invention involves the combined administration of an anti-ErbB2 antibody (or antibodies) and one or more chemotherapeutic agents or growth inhibitory agents, including coadministration of cocktails of different chemotherapeutic agents. Preferred chemotherapeutic agents include taxanes (such as paclitaxel and docetaxel) and/or anthracycline antibiotics. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service Ed., M. C. Perry, Williams & Wilkins, Baltimore, Md. (1992).

The antibody may be combined with an anti-hormonal compound; e.g., an anti-estrogen compound such as tamoxifen; an anti-progesterone such as onapristone (see, EP 616 812); or an anti-androgen such as flutamide, in dosages known for such molecules. Where the cancer to be treated is hormone independent cancer, the patient may previously have been subjected to anti-hormonal therapy and, after the cancer becomes hormone independent, the anti-ErbB2 antibody (and optionally other agents as described herein) may be administered to the patient.

Sometimes, it may be beneficial to also coadminister a cardioprotectant (to prevent or reduce myocardial dysfunction associated with the therapy) or one or more cytokines to the patient. One may also coadminister an EGFR-targeted drug or an anti-angiogenic agent. In addition to the above therapeutic regimes, the patient may be subjected to surgical removal of cancer cells and/or radiation therapy.

The anti-ErbB2 antibodies herein may also be combined with an EGFR-targeted drug such as those discussed above in the definitions section resulting in a complementary, and potentially synergistic, therapeutic effect.

Suitable dosages for any of the above coadministered agents are those presently used and may be lowered due to the combined action (synergy) of the agent and anti-ErbB2 antibody.

For the prevention or treatment of disease, the appropriate dosage of antibody will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1–20 mg/kg) of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. The preferred dosage of the antibody will be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, e.g. about six doses of the anti-ErbB2 antibody). An initial higher loading dose, followed by one or more lower doses may be administered. An exemplary dosing regimen comprises administering an initial loading dose of about 4 mg/kg, followed by a weekly maintenance dose of about 2 mg/kg of the anti-ErbB2 antibody. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

Aside from administration of the antibody protein to the patient, the present application contemplates administration of the antibody by gene therapy. Such administration of nucleic acid encoding the antibody is encompassed by the expression "administering a therapeutically effective amount of an antibody". See, for example, WO96/07321 published Mar. 14, 1996 concerning the use of gene therapy to generate intracellular antibodies.

There are two major approaches to getting the nucleic acid (optionally contained in a vector) into the patient's cells; in vivo and ex vivo. For in vivo delivery the nucleic acid is injected directly into the patient, usually at the site where the antibody is required. For ex vivo treatment, the patient's cells are removed, the nucleic acid is introduced into these isolated cells and the modified cells are administered to the patient either directly or, for example, encapsu-

lated within porous membranes which are implanted into the patient (see, e.g. U.S. Pat. Nos. 4,892,538 and 5,283,187). There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells in vitro, or in vivo in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells in vitro include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. A commonly used vector for ex vivo delivery of the gene is a retrovirus.

The currently preferred in vivo nucleic acid transfer techniques include transfection with viral vectors (such as adenovirus, Herpes simplex I virus, or adeno-associated virus) and lipid-based systems (useful lipids for lipid-mediated transfer of the gene are DOTMA, DOPE and DC-Chol, for example). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, e.g. capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu et al., *J. Biol. Chem.* 262:4429–4432 (1987); and Wagner et al., *Proc. Natl. Acad. Sci. USA* 87:3410–3414 (1990). For review of the currently known gene marking and gene therapy protocols see Anderson et al., *Science* 256:808–813 (1992). See also WO 93/25673 and the references cited therein.

VI. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-ErbB2 antibody. The label or package insert indicates that the composition is used for treating the condition of choice, such as cancer. In one embodiment, the label or package inserts indicates that the composition comprising the antibody which binds ErbB2 can be used to treat cancer which expresses an ErbB receptor selected from the group consisting of epidermal growth factor receptor (EGFR), ErbB3 and ErbB4, preferably EGFR. In addition, the label or package insert may indicate that the patient to be treated is one having cancer characterized by excessive activation of an ErbB receptor selected from EGFR, ErbB 3 or ErbB4. For example, the cancer may be one which overexpresses one of these receptors and/or which overexpresses an ErbB ligand (such as TGF-α). The label or package insert may also indicate that the composition can be used to treat cancer, wherein the cancer is not characterized by overexpression of the ErbB2 receptor. For example, whereas the present package insert for HERCEPTIN® indicates that the antibody is used to treat patients with metastatic breast cancer whose tumors overexpress the ErbB2 protein, the package insert herein may indicate that the antibody or composition is used to treat cancer regardless of the extent of ErbB2 overexpression. In other embodiments, the package insert may indicate that the antibody or composition can be used to treat breast cancer (e.g. metastatic breast cancer); hormone independent cancer; prostate cancer, (e.g. androgen independent prostate cancer); lung cancer (e.g. non-small cell lung cancer); colon, rectal or colorectal cancer; or any of the other diseases or disorders disclosed herein. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a first antibody which binds ErbB2 and inhibits growth of cancer cells which overexpress ErbB2; and (b) a second container with a composition contained therein, wherein the composition comprises a second antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor. The article of manufacture in this embodiment of the invention may further comprises a package insert indicating that the first and second antibody compositions can be used to treat cancer. Moreover, the package insert may instruct the user of the composition (comprising an antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor) to combine therapy with the antibody and any of the adjunct therapies described in the preceding section (e.g. a chemotherapeutic agent, an EGFR-targeted drug, an anti-angiogenic agent, an anti-hormonal compound, a cardioprotectant and/or a cytokine). Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

VII. Non-Therapeutic Uses for the Anti-ErbB2 Antibody

The antibodies (e.g. the humanized anti-ErbB2 antibodies) of the invention have further non-therapeutic applications.

For example, the antibodies may be used as affinity purification agents. In this process, the antibodies are immobilized on a solid phase such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody is contacted with a sample containing the ErbB2 protein (or fragment thereof) to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the ErbB2 protein, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent, such as glycine buffer, pH 5.0, that will release the ErbB2 protein from the antibody.

Anti-ErbB2 antibodies may also be useful in diagnostic assays for ErbB2 protein, e.g., detecting its expression in specific cells, tissues, or serum.

For diagnostic applications, the antibody typically will be labeled with a detectable moiety. Numerous labels are available which can be generally grouped into the following categories;

(a) Radioisotopes, such as ^{35}S, ^{14}C, ^{125}I, ^{3}H, and ^{131}I The antibody can be labeled with the radioisotope using the techniques described in Current Protocols in Immunology, Volumes 1 and 2, Coligen et al., Ed. Wiley-Interscience, New York, N.Y., Pubs. (1991) for example and radioactivity can be measured using scintillation counting.

(b) Fluorescent labels such as rare earth chelates (europium chelates) or fluorescein and its derivatives,

rhodamine and its derivatives, dansyl, Lissamine, phycoerythrin and Texas Red are available. The fluorescent labels can be conjugated to the antibody using the techniques disclosed in Current Protocols in Immunology, supra, for example. Fluorescence can be quantified using a fluorimeter.

(c) Various enzyme-substrate labels are available and U.S. Pat. No. 4,275,149 provides a review of some of these. The enzyme generally catalyzes a chemical alteration of the chromogenic substrate which can be measured using various techniques. For example, the enzyme may catalyze a color change in a substrate, which can be measured spectrophotometrically. Alternatively, the enzyme may alter the fluorescence or chemiluminescence of the substrate. Techniques for quantifying a change in fluorescence are described above. The chemiluminescent substrate becomes electronically excited by a chemical reaction and may then emit light which can be measured (using a chemiluminometer, for example) or donates energy to a fluorescent acceptor. Examples of enzymatic labels include luciferases (e.g., firefly luciferase and bacterial luciferase; U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Techniques for conjugating enzymes to antibodies are described in O'Sullivan et al., Methods for the Preparation of Enzyme-Antibody Conjugates for use in Enzyme Immunoassay, in *Methods in Enzym.* (ed J. Langone & H. Van Vunakis), Academic press, New York, 73:147–166 (1981).

Examples of enzyme-substrate combinations include, for example:

(i) Horseradish peroxidase (HRPO) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (e.g., orthophenylene diamine (OPD) or 3,3',5,5'-tetramethyl benzidine hydrochloride (TMB));

(ii) alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and

(iii) β-D-galactosidase (β-D-Gal) with a chromogenic substrate (e.g., p-nitrophenyl-β-D-galactosidase) or fluorogenic substrate 4-methylumbelliferyl-β-D-galactosidase.

Numerous other enzyme-substrate combinations are available to those skilled in the art. For a general review of these, see U.S. Pat. Nos. 4,275,149 and 4,318,980.

Sometimes, the label is indirectly conjugated with the antibody. The skilled artisan will be aware of various techniques for achieving this. For example, the antibody can be conjugated with biotin and any of the three broad categories of labels mentioned above can be conjugated with avidin, or vice versa. Biotin binds selectively to avidin and thus, the label can be conjugated with the antibody in this indirect manner. Alternatively, to achieve indirect conjugation of the label with the antibody, the antibody is conjugated with a small hapten (e.g., digoxin) and one of the different types of labels mentioned above is conjugated with an anti-hapten antibody (e.g., anti-digoxin antibody). Thus, indirect conjugation of the label with the antibody can be achieved.

In another embodiment of the invention, the anti-ErbB2 antibody need not be labeled, and the presence thereof can be detected using a labeled antibody which binds to the ErbB2 antibody.

The antibodies of the present invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, Monoclonal Antibodies: *A Manual of Techniques*, pp. 147–158 (CRC Press, Inc. 1987).

For immunohistochemistry, the tumor sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin, for example.

The antibodies may also be used for in vivo diagnostic assays. Generally, the antibody is labeled with a radio nuclide (such as ^{111}In, ^{99}Tc, ^{14}C, ^{131}I, ^{125}I, ^{3}H, ^{32}P or ^{35}S) so that the tumor can be localized using immunoscintiography. As a matter of convenience, the antibodies of the present invention can be provided in a kit, i.e., a packaged combination of reagents in predetermined amounts with instructions for performing the diagnostic assay. Where the antibody is labeled with an enzyme, the kit will include substrates and cofactors required by the enzyme (e.g., a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives may be included such as stabilizers, buffers (e.g., a block buffer or lysis buffer) and the like. The relative amounts of the various reagents may be varied widely to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

VIII. Deposit of Materials

The following hybridoma cell lines have been deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Va. 20110-2209, USA (ATCC):

Antibody Designation	ATCC No.	Deposit Date
7C2	ATCC HB-12215	Oct. 17, 1996
7F3	ATCC HB-12216	Oct. 17, 1996
4D5	ATCC CRL 10463	May 24, 1990
2C4	ATCC HB-12697	Apr. 8, 1999

Further details of the invention are illustrated by the following non-limiting Examples. The disclosures of all citations in the specification are expressly incorporated herein by reference.

EXAMPLE 1

Production and Characterization of Monoclonal Antibody 2C4

The murine monoclonal antibodies 2C4, 7F3 and 4D5 which specifically bind the extracellular domain of ErbB2 were produced as described in Fendly et al., *Cancer Research* 50:1550–1558 (1990). Briefly, NIH 3T3/HER2-3_{400} cells (expressing approximately 1×10 ErbB2 molecules/cell) produced as described in Hudziak et al *Proc. Natl. Acad. Sci.* (USA) 84:7158–7163 (1987) were harvested with phosphate buffered saline (PBS) containing 25 mM EDTA and used to immunize BALB/c mice. The mice were given injections i.p. of 10^7 cells in 0.5 ml PBS on weeks 0, 2, 5 and 7. The mice with antisera that immunoprecipitated ^{32}P-labeled ErbB2 were given i.p. injections of a wheat germ agglutinin-Sepharose (WGA) purified ErbB2 membrane extract on weeks 9 and 13. This was followed by an i.v. injection of 0.1 ml of the ErbB2 preparation and the splenocytes were fused with mouse myeloma line X63-Ag8.653.

Hybridoma supernatants were screened for ErbB2-binding by ELISA and radioimmunoprecipitation.

The ErbB2 epitopes bound by monoclonal antibodies 4D5, 7F3 and 2C4 were determined by competitive binding

analysis (Fendly et al. *Cancer Research* 50:1550–1558 (1990)). Cross-blocking studies were done on antibodies by direct fluorescence on intact cells using the PANDEX™ Screen Machine to quantitate fluorescence. Each monoclonal antibody was conjugated with fluorescein isothiocyanate (FITC), using established procedures (Wofsy et al. *Selected Methods in Cellular Immunology, p.* 287, Mishel and Schiigi (eds.) San Francisco: W. J. Freeman Co. (1980)). Confluent monolayers of NTH 3T3/HER2-3_{400} cells were trypsinized, washed once, and resuspended at 1.75×10^6 cell/ml in cold PBS containing 0.5% bovine serum albumin (BSA) and 0.1% NaN_3. A final concentration of 1% latex particles (IDC, Portland, Oreg.) was added to reduce clogging of the PANDEX™ plate membranes. Cells in suspension, 20 μl, and 20 μl of purified monoclonal antibodies (100 μg/ml to 0.1 μg/ml) were added to the PANDEX™ plate wells and incubated on ice for 30 minutes. A predetermined dilution of FITC-labeled monoclonal antibodies in 20 μl was added to each well, incubated for 30 minutes, washed, and the fluorescence was quantitated by the PANDEX™. Monoclonal antibodies were considered to share an epitope if each blocked binding of the other by 50% or greater in comparison to an irrelevant monoclonal antibody control. In this experiment, monoclonal antibodies 4D5, 7F3 and 2C4 were assigned epitopes 1, G/F and F, respectively.

The growth inhibitory characteristics of monoclonal antibodies 2C4, 7F3 and 4D5 were evaluated using the breast tumor cell line, SK-BR-3 (see Hudziak et al. *Molec. Cell. Biol.* 9(3):1165–1172 (1989)). Briefly, SK-BR-3 cells were detached by using 0.25% (vol/vol) trypsin and suspended in complete medium at a density of 4×10^5 cells per ml. Aliquots of 100 μl (4×10^4 cells) were plated into 96-well microdilution plates, the cells were allowed to adhere, and 100 μl of media alone or media containing monoclonal antibody (final concentration 51g/ml) was then added. After 72 hours, plates were washed twice with PBS (pH 7.5), stained with crystal violet (0.5% in methanol), and analyzed for relative cell proliferation as described in Sugarman et al. *Science* 230:943–945 (1985). Monoclonal antibodies 2C4 and 7F3 inhibited SK-BR-3 relative cell proliferation by about 20% and about 38%, respectively, compared to about 56% inhibition achieved with monoclonal antibody 4D5.

Monoclonal antibodies 2C4, 4D5 and 7F3 were evaluated for their ability to inhibit HRG-stimulated tyrosine phosphorylation of proteins in the M_r 180,000 range from whole-cell lysates of MCF7 cells (Lewis et al. *Cancer Research* 56:1457–1465 (1996)). MCF7 cells are reported to express all known ErbB receptors, but at relatively low levels. Since ErbB2, ErbB3, and ErbB4 have nearly identical molecular sizes, it is not possible to discern which protein is becoming tyrosine phosphorylated when whole-cell lysates are evaluated by Western blot analysis.

However, these cells are ideal for HRG tyrosine phosphorylation assays because under the assay conditions used, in the absence of exogenously added HRG, they exhibit low to undetectable levels of tyrosine phosphorylation proteins in the M_r 180,000 range.

MCF7 cells were plated in 24-well plates and monoclonal antibodies to ErbB2 were added to each well and incubated for 30 minutes at room temperature; then $rHRG\beta1_{177-244}$ was added to each well to a final concentration of 0.2 nM, and the incubation was continued for 8 minutes. Media was carefully aspirated from each well, and reactions were stopped by the addition of 100 μl of SDS sample buffer (5% SDS, 25 mM DTT, and 25 mM Tris-HCl, pH 6.8). Each sample (25 μl) was electrophoresed on a 4–12% gradient gel (Novex) and then electrophoretically transferred to polyvinylidene difluoride membrane. Antiphosphotyrosine (4G10, from UBI, used at 1 μg/ml) immunoblots were developed, and the intensity of the predominant reactive band at M_r-180,000 was quantified by reflectance densitometry, as described previously (Holmes et al. *Science* 256:1205–1210 (1992); Sliwkowski et al. *J. Biol. Chem.* 269:14661–14665 (1994)).

Monoclonal antibodies 2C4, 7F3, and 4D5, significantly inhibited the generation of a HRG-induced tyrosine phosphorylation signal at M_r 180,000. In the absence of HRG, none of these antibodies were able to stimulate tyrosine phosphorylation of proteins in the M_r 180,000 range. Also, these antibodies do not cross-react with EGFR (Fendly et al. *Cancer Research* 50:1550–1558 (1990)), ErbB3, or ErbB4. Antibodies 2C4 and 7F3 significantly inhibited HRG stimulation of p180 tyrosine phosphorylation to <25% of control. Monoclonal antibody 4D5 was able to block HRG stimulation of tyrosine phosphorylation by ~50%. FIG. 2A shows dose-response curves for 2C4 or 7F3 inhibition of HRG stimulation of p180 tyrosine phosphorylation as determined by reflectance densitometry. Evaluation of these inhibition curves using a 4-parameter fit yielded an IC_{50} of 2.8±0.7 nM and 29.0±4.1 nM for 2C4 and 7F3, respectively.

Inhibition of HRG binding to MCF7 breast tumor cell lines by anti-ErbB2 antibodies was performed with monolayer cultures on ice in a 24-well-plate format (Lewis et al. *Cancer Research* 56:1457–1465 (1996)). Anti-ErbB2 monoclonal antibodies were added to each well and incubated for 30 minutes. ^{125}I-labeled $rHRG\beta1_{177-224}$ (25 pm) was added, and the incubation was continued for 4 to 16 hours. FIG. 2B provides dose-response curves for 2C4 or 7F3 inhibition of HRG binding to MCF7 cells. Varying concentrations of 2C4 or 7F3 were incubated with MCF7 cells in the presence of 125I-labeled rHRGβ1, and the inhibition curves are shown in FIG. 2B. Analysis of these data yielded an IC_{50} of 2.4±0.3 nM and 19.0±7.3 nM for 2C4 and 7F3, respectively. A maximum inhibition of ~74% for 2C4 and 7F3 were in agreement with the tyrosine phosphorylation data.

To determine whether the effect of the anti-ErbB2 antibodies observed on MCF7 cells was a general phenomenon, human tumor cell lines were incubated with 2C4 or 7F3 and the degree of specific ^{125}I-labeled rHRGβ1 binding was determined (Lewis et al. *Cancer Research* 56:1457-1465 (1996)). The results from this study are shown in FIG. 3. Binding of ^{121}I-labeled rHRGβ1 could be significantly inhibited by either 2C4 or 7F3 in all cell lines, with the exception of the breast cancer cell line MDA-MB-468, which has been reported to express little or no ErbB2. The remaining cell lines are reported to express ErbB2, with the level of ErbB2 expression varying widely among these cell lines. In fact, the range of ErbB2 expression in the cell lines tested varies by more than 2 orders of magnitude. For example, BT-20, MCF7, and Caov3 express ~10^4 ErbB2 receptors/cell, whereas BT474 and SK-BR-3 express ~10^6 ErbB2 receptors/cell. Given the wide range of ErbB2 expression in these cells and the data above, it was concluded that the interaction between ErbB2 and ErbB3 or ErbB4, was itself a high-affinity interaction that takes place on the surface of the plasma membrane.

The growth inhibitory effects of monoclonal antibodies 2C4 and 4D5 on MDA-MB-175 and SK-BR-3 cells in the presence or absence of exogenous rHRGβ1 was assessed (Schaefer et al. *Oncogene* 15:1385–1394 (1997)). ErbB2 levels in MDA-MB-175 cells are 4–6 times higher than the level found in normal breast epithelial cells and the ErbB2–ErbB4 receptor is constitutively tyrosine phospho-

rylated in MDA-MB-175 cells. MDA-MB-175 cells were treated with an anti-ErbB2 monoclonal antibodies 2C4 and 4D5 (10 μg/mL) for 4 days. In a crystal violet staining assay, incubation with 2C4 showed a strong growth inhibitory effect on this cell line (FIG. 4A). Exogenous HRG did not significantly reverse this inhibition. On the other hand 2C4 revealed no inhibitory effect on the ErbB2 overexpressing cell line SK-BR-3 (FIG. 4B). Monoclonal antibody 2C4 was able to inhibit cell proliferation of MDA-MB-175 cells to a greater extent than monoclonal antibody 4D5, both in the presence and absence of exogenous HRG. Inhibition of cell proliferation by 4D5 is dependent on the ErbB2 expression level (Lewis et al. *Cancer Immunol. Immunother.* 37:255–263 (1993)). A maximum inhibition of 66% in SK-BR-3 cells could be detected (FIG. 4B). However this effect could be overcome by exogenous HRG.

EXAMPLE 2

HRG Dependent Association of ErbB2 with ErbB3 is Blocked by Monoclonal Antibody 2C4

The ability of ErbB3 to associate with ErbB2 was tested in a co-immunoprecipitation experiment. 1.0×10^6 MCF7 or SK-BR-3 cells were seeded in six well tissue culture plates in 50:50 DMEM/Ham's F12 medium containing 10% fetal bovine serum (FBS) and 10 mM HEPES, pH 7.2 (growth medium), and allowed to attach overnight. The cells were starved for two hours in growth medium without serum prior to beginning the experiment

The cells were washed briefly with phosphate buffered saline (PBS) and then incubated with either 100 nM of the indicated antibody diluted in 0.2% w/v bovine serum albumin (BSA), RPMI medium, with 10 mM HEPES, pH 7.2 (binding buffer), or with binding buffer alone (control). After one hour at room temperature, HRG was added to a final concentration of 5 nM to half the wells (+). A similar volume of binding buffer was added to the other wells (–). The incubation was continued for approximately 10 minutes.

Supernatants were removed by aspiration and the cells were lysed in RPMI, 10 mM HEPES, pH 7.2, 1.0% v/v TRITON X-100™, 1.0% w/v CHAPS (lysis buffer), containing 0.2 mM PMSF, 10 μg/ml leupeptin, and 10 TU/ml aprotinin. The lysates were cleared of insoluble material by centrifugation.

ErbB2 was immunoprecipitated using a monoclonal antibody covalently coupled to an affinity gel (Affi-Prep 10, Bio-Rad). This antibody (Ab-3, Oncogene Sciences) recognizes a cytoplasmic domain epitope. Immunoprecipitation was performed by adding 10 μl of gel slurry containing approximately 8.5 μg of immobilized antibody to each lysate, and the samples were allowed to mix at room temperature for two hours. The gels were then collected by centrifugation. The gels were washed batchwise three times with lysis buffer to remove unbound material. SDS sample buffer was then added and the samples were heated briefly in a boiling water bath.

Supernatants were run on 4–12% polyacrylamide gels and electroblotted onto nitrocellulose membranes. The presence of ErbB3 was assessed by probing the blots with a polyclonal antibody against a cytoplasmic domain epitope thereof (c-17, Santa Cruz Biotech). The blots were visualized using a chemiluminescent substrate (ECL, Amersham).

As shown in the control lanes of FIGS. 5A and 5B, for MCF7 and SK-BR-3 cells, respectively, ErbB3 was present in an ErbB2 immunoprecipitate only when the cells were stimulated with HRG. If the cells were first incubated with monoclonal antibody 2C4, the ErbB3 signal was abolished in MCF7 cells (FIG. 5A, lane 2C4+) or substantially reduced in SK-BR-3 cells (FIG. 5B, lane 2C4+). As shown in FIGS. 5A–B, monoclonal antibody 2C4 blocks heregulin dependent association of ErbB3 with ErbB2 in both MCF7 and SK-BR-3 cells substantially more effectively than HERCEPTIN®. Preincubation with HERCEPTIN® decreased the ErbB3 signal in MCF7 lysates but had little or no effect on the amount of ErbB3 co-precipitated from SK-BR-3 lysates. Preincubation with an antibody against the EGF receptor (Ab-1, Oncogene Sciences) had no effect on the ability of ErbB3 to co-immunoprecipitate with ErbB2 in either cell line.

EXAMPLE 3

Humanized 2C4 Antibodies

The variable domains of murine monoclonal antibody 2C4 were first cloned into a vector which allows production of a mouse/human chimeric Fab fragment. Total RNA was isolated from the hybridoma cells using a Stratagene RNA extraction kit following manufacturer's protocols. The variable domains were amplified by RT-PCR, gel purified, and inserted into a derivative of a pUC119-based plasmid containing a human kappa constant domain and human C_H1 domain as previously described (Carter et al. *PNAS* (USA) 89:4285 (1992); and U.S. Pat. No. 5,821,337). The resultant plasmid was transformed into *E. coli* strain 16C9 for expression of the Fab fragment. Growth of cultures, induction of protein expression, and purification of Fab fragment were as previously described (Werther et al. *J. Immunol.* 157:4986–4995 (1996); Presta et al. *Cancer Research* 57:4593–4599 (1997)).

Purified chimeric 2C4 Fab fragment was compared to the murine parent antibody 2C4 with respect to its ability to inhibit ^{125}1-HRG binding to MCF7 cells and inhibit rHRG activation of p180 tyrosine phosphorylation in MCF7 cells. As shown in FIG. 6A, the chimeric 2C4 Fab fragment is very effective in disrupting the formation of the high affinity ErbB2–ErbB3 binding site on the human breast cancer cell line, MCF7. The relative IC_{50} value calculated for intact murine 2C4 is 4.0±0.4 nM, whereas the value for the Fab fragment is 7.7±1.1 nM. As illustrated in FIG. 6B, the monovalent chimeric 2C4 Fab fragment is very effective in disrupting HRG-dependent ErbB2–ErbB3 activation. The IC_{50} value calculated for intact murine monoclonal antibody 2C4 is 6.0±2 nM, whereas the value for the Fab fragment is 15.0 t 2 nM.

DNA sequencing of the chimeric clone allowed identification of the CDR residues (Kabat et al, *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) (FIGS. 7A and B). Using oligonucleotide site-directed mutagenesis, all six of these CDR regions were introduced into a complete human framework (V_L kappa subgroup I and V_H subgroup III) contained on plasmid VX4 as previously described (Presta et al., *Cancer Research* 57: 4593–4599 (1997)). Protein from the resultant "CDR-swap" was expressed and purified as above. Binding studies were performed to compare the two versions. Briefly, a NUNC MAXISORP™ plate was coated with 1 microgram per ml of ErbB2 extracellular domain (ECD; produced as described in WO 90/14357) in 50 mM carbonate buffer, pH 9.6, overnight at 4° C., and then blocked with ELISA diluent (0.5% BSA, 0.05% polysorbate 20, PBS) at room temperature for 1 hour. Serial dilutions of samples in ELISA diluent were incubated on the plates for 2 hours. After washing, bound

Fab fragment was detected with biotinylated murine anti-human kappa antibody (ICN 634771) followed by streptavidin-conjugated horseradish peruxidase (Sigma) and using 3,3',5,5'-tetramethyl benzidine (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) as substrate. Absorbance was read at 450 nm. As shown in FIG. 8A, all binding was lost on construction of the CDR-swap human Fab fragment.

To restore binding of the humanized Fab, mutants were constructed using DNA from the CDR-swap as template. Using a computer generated model (FIG. 9), these mutations were designed to change human framework region residues to their murine counterparts at positions where the change might affect CDR conformations or the antibody-antigen interface. Mutants are shown in Table 2.

TABLE 2

Designation of Humanized 2C4 FR Mutations

Mutant no.	Framework region (FR) substitutions
560	ArgH71Val
561	AspH73Arg
562	ArgH71Val, AspH73Arg
568	ArgH71Val, AspH73Arg, AlaH49Gly
569	ArgH71Val, AspH73Arg, PheH67Ala
570	ArgH71Val, AspH73Arg, AsnH76Arg
571	ArgH71Val, AspH73Arg, LeuH78Val
574	ArgH71Val, AspH73Arg, IleH69Leu
56869	ArgH71Val, AspH73Arg, AlaH49Gly, PheH67Ala

Binding curves for the various mutants are shown in FIGS. 8A–C. Humanized Fab version 574, with the changes ArgH71Val, AspH73Arg and IleH69Leu, appears to have binding restored to that of the original chimeric 2C4 Fab fragment. Additional FR and/or CDR residues, such as L2, L54, L55, L56, H35 and/or H48, may be modified (e.g. substituted as follows—IleL2Thr; ArgL54Leu; TyrL55Glu; ThrL56Ser; AspH35Ser; and ValH48Ile) in order to further refine or enhance binding of the humanized antibody. Alternatively, or additionally, the humanized antibody may be affinity matured (see above) in order to further improve or refine its affinity and/or other biological activities.

Humanized 2C4 version 574 was affinity matured using a phage-display method. Briefly, humanized 2C4.574 Fab was cloned into a phage display vector as a geneIII fusion. When phage particles are induced by infection with M13KO7 helper phage, this fusion allows the Fab to be displayed on the N-terminus of the phage tail-fiber protein, geneIII (Baca et al. *J Biol Chem.* 272:10678 (1997)).

Individual libraries were constructed for each of the 6 CDRs identified above. In these libraries, the amino acids in the CDRs which were identified using a computer generated model (FIG. 9) as being potentially significant in binding to ErbB2 were randomized using oligos containing "NNS" as their codons. The libraries were then panned against ErbB2 ECD coated on NUNC MAXISORP™ plates with 3% dry milk in PBS with 0.2% TWEEN 20® (MPBST) used in place of all blocking solutions. In order to select for phage with affinities higher than that of 2C4.574, in panning rounds 3, 4, and 5, soluble ErbB2 ECD or soluble Fab 2C4.574 was added during the wash steps as competitor. Wash times were extended to 1 hour at room temperature.

After 5 rounds of panning, individual clones were again analyzed by phage-ELISA. Individual clones were grown in Costar 96-well U-bottomed tissue culture plates, and phage were induced by addition of helper phage. After overnight growth, *E. coli* cells were pelleted, and the phage-containing supernates were transfered to 96-well plates where the phage were blocked with MPBST for 1 hr at room temperature. NUNC MAXISORP™ plates coated with ErbB2 ECD were also blocked with MPBST as above. Blocked phage were incubated on the plates for 2 hours. After washing, bound phage were detected using horseradish-peroxidase-conjugated anti-M 13 monoclonal antibody (Amersham Pharmacia Biotech, Inc. 27-9421-01) diluted 1:5000 in MPBST, followed by 3,3',5,5',-tetramethyl benzidine as substrate. Absorbance was read at 450 nm.

The 48 clones from each library which gave the highest signals were DNA sequenced. Those clones whose sequences occurred the most frequently were subcloned into the vector described above which allows expression of soluble Fabs. These Fabs were induced, proteins purified and the purified Fabs were analyzed for binding by ELISA as described above and the binding was compared to that of the starting humanized 2C4.574 version.

After interesting mutations in individual CDRs were identified, additional mutants which were various combinations of these were constructed and tested as above. Mutants which gave improved binding relative to 574 are described in Table 3.

TABLE 3

Designation of mutants derived from affinity maturation of 2C4.574

Mutant Name	Change from 574	Mutant/574*
H3.A1	serH99trp, metH34leu	0.380
L2.F5	serL50trp, tyrL53gly, metH34leu	0.087
H1.3.B3	thrH28gln, thrH30ser, metH34leu	0.572
L3.G6	tyrL92pro, ileL93lys, metH34leu	0.569
L3.G11	tyrL92ser, ileL93arg, tyrL94gly, metH34leu	0.561
L3.29	tyrL92phe, tyrL96asn, metH34leu	0.552
L3.36	tyrL92phe, tyrL94leu, tyrL96pro, metH34leu	0.215
654	serL50trp, metH34leu	0.176
655	metH34ser	0.542
659	serL50trp, metH34ser	0.076
L2.F5.H3.A1	serL50trp, tyrL53gly, metH34leu, serH99trp	0.175
L3G6.H3.A1	tyrL92pro, ileL93lys, metH34leu, serH99trp	0.218
H1.3.B3.H3.A1	thrH28gln, thrH30ser, metH34leu, serH99trp	0.306
L3.G11.H3.A1	tyrL92ser, ileL93arg, tyrL94gly, metH34leu, serH99trp	0.248
654.H3.A1	serL50trp, metH34leu, serH99trp	0.133
654.L3.G6	serL50trp, metH34leu, tyrL92pro, ileL93lys	0.213
654.L3.29	serL50trp, metH34leu, tyrL92phe, tyrL96asn	0.236
654.L3.36	serL50trp, metH35leu, tyrL92phe, tyrL94leu, tyrL96pro	0.141

*Ratio of the amount of mutant needed to give the mid-OD of the standard curve to the amount of 574 needed to give the mid-OD of the standard curve in an Erb2-ECD ELISA. A number less than 1.0 indicates that the mutant binds Erb2 better than 574 binds.

The following mutants have also been constructed, and are currently under evaluation:

659.L3.G6	serL50trp, metH34ser, tyrL92pro, ileL93lys
659.L3.G11	serL50trp, metH34ser, tyrL92ser, ileL93arg, tyrL94gly
659.L3.29	serL50trp, metH34ser, tyrL92phe, tyrL96asn
659.L3.36	serL50trp, metH34ser, tyrL92phe, tyrL94leu, tyrL96pro
L2F5.L3G6	serL50trp, tyrL53gly, metH34leu, tyrL92pro, ileL93lys
L2F5.L3G11	serL50trp, tyrL53gly, metH34leu, tyrL92ser, ileL93arg, tyrL94gly

-continued

L2F5.L29	serL50trp, tyrL53gly, metH34leu, tyrL92phe, tyrL96asn
L2F5.L36	serL50trp, tyrL53gly, metH34leu, tyrL92phe, tyrL94leu, tyrL96pro
L2F5.L3G6.655	serL50trp, tyrL53gly, metH35ser, tyrL92pro, ileL93lys
L2F5.L3G11.655	serL50trp, tyrL53gly, metH34ser, tyrL92ser, ileL93arg, tyrL94gly
L2F5.L29.655	serL50trp, tyrL53gly, metH34ser, tyrL92phe, tyrL96asn
L2F5.L36.655	serL50trp, tyrL53gly, metH34ser, tyrL92phe, tyrL94leu, tyrL96pro

The following mutants, suggested by a homology scan, are currently being constructed:

678	thrH30ala
679	thrH30ser
680	lysH64arg
681	leuH96val
682	thrL97ala
683	thrL97ser
684	tyrL96phe
685	tyrL96ala
686	tyrL91phe
687	thrL56ala
688	glnL28ala
689	glnL28glu

The preferred amino acid at H34 would be methionine. A change to leucine might be made if there were found to be oxidation at this position.

AsnH52 and asnH53 were found to be strongly preferred for binding. Changing these residues to alanine or aspartic acid dramatically decreased binding.

An intact antibody comprising the variable light and heavy domains of humanized version 574 with a human IgG1 heavy chain constant region has been prepared (see U.S. Pat. No. 5,821,337). The intact antibody is produced by Chinese Hamster Ovary (CHO) cells. That molecule is designated rhuMAb 2C4 herein.

EXAMPLE 4

Monoclonal Antibody 2C4 Blocks EGF, TGF-α or HRG Mediated Activation of MAPK

Many growth factor receptors signal through the mitogen-activated protein kinase (MAPK) pathway. These dual specificity kinases are one of the key endpoints in signal transduction pathways that ultimately triggers cancer cells to divide. The ability of monoclonal antibody 2C4 or HERCEPTIN® to inhibit EGF, TGF-α or HRG activation of MAPK was assessed in the following way.

MCF7 cells (10^5 cells/well) were plated in serum containing media in 12-well cell culture plates. The next day, the cell media was removed and fresh media containing 0.1% serum was added to each well. This procedure was then repeated the following day and prior to assay the media was replaced with serum-free binding buffer (Jones et al. *J. Biol. Chem.* 273:11667–74(1998); and Schaefer et al. *J. Biol. Chem.* 274:859–66(1999)). Cells were allowed to equilibrate to room temperature and then incubated for 30 minutes with 0.5 mL of 200 nM HERCEPTIN® or monoclonal antibody 2C4. Cells were then treated with 1 nM EGF, 1 nM TGF-α or 0.2 nM HRG for 15 minutes. The reaction was stopped by aspirating the cell medium and then adding 0.2 mL SDS-PAGE sample buffer containing 1% DTT.MAPK activation was assessed by Western blotting using an anti-active MAPK antibody (Promega) as described previously (Jones et al. *J. Biol. Chem.* 273:11667–74 (1998)).

As shown in FIG. **10**, monoclonal antibody 2C4 significantly blocks EGF, TGF-α and HRG mediated activation of MAPK to a greater extent than HERCEPTIN®. These data suggest that monoclonal antibody 2C4 binds to a surface of ErbB2 that is used for its association with either EGFR or ErbB3 and thus prevents the formation of the signaling receptor complex.

Monoclonal antibody 2C4 was also shown to inhibit heregulin (HRG)-dependent Akt activation. Activation of the PI3 kinase signal transduction pathway is important for cell survival (Carraway et al *J. Biol. Chem.* 270:7111–6 (1995)). In tumor cells, PI3 kinase activation may play a role in the invasive phenotype (Tan et al. *Cancer Reearch.* 59: 1620–1625, (1999)). The survival pathway is primarily mediated by the serine/threonine kinase AKT (Bos et al *Trends Biochem Sci.* 20: 441–442 (1995). Complexes formed between ErbB2 and either ErbB3 or EGFR can initiate these pathways in response to heregulin or EGF, respectively (Olayioye et al. *Mol. & Cell. Biol.* 18: 5042–51 (1998); Karunagaran et al., *EMBO Journal.* 15:254–264 (1996); and Krymskaya et al *Am. J. Physiol.* 276: L246–55 (1999)). Incubation of MCF7 breast cancer cells with 2C4 inhibits heregulin-mediated AKT activation. Moreover, the basal level of AKT activation present in the absence of heregulin addition is further reduced by the addition of 2C4. These data suggest that 2C4 may inhibit ErbB ligand-activation of PI3 kinase and that this inhibition may lead to apoptosis. The increased sensitivity to apoptosis may manifest in a greater sensitivity of tumor cells to the toxic effects of chemotherapy.

Thus, monoclonal antibody 2C4 inhibits ligand initiated ErbB signaling through two major signal transduction pathways—MAP Kinase (a major proliferative pathway) and P 13 kinase (a major survival/anti-apoptotic pathway).

EXAMPLE 5

Combination of Monoclonal Antibody 2C4 and HERCEPTIN® in vivo

A xenograft model using the lung adenocarcinoma cell line, Calu-3, was used to assess the efficacy of anti-HER2 monoclonal antibodies, either alone or in combination, to suppress tumor growth. Female NCR nude mice were inoculated subcutaneously with 20×10^6 cells in 0.1 mL. Tumor measurements were taken twice per week and when tumor nodules reached a volume of 100 mm^3, animals were randomized to 7 treatment groups. The treatment groups were:

(a) control monoclonal antibody, MAb 1766;

(b) HERCEPTIN®, 10 mg/kg;

(c) monoclonal antibody 7C2, 10 mg/kg;

(d) monoclonal antibody 2C4, 10 mg/kg;

(e) HERCEPTIN® and 7C2, each at 10 mg/kg;

(f) HERCEPTIN® and 2C4, each at 10 mg/kg; and

(g) Monoclonal antibodies 2C4 and 7C2, each at 10 mg/kg.

Animals were treated twice per week until day 24. Tumor volumes were measured twice per week until day 38.

As shown in the bar graph in FIG. **11**, treatment of the Calu-3 tumor-bearing mice with 2C4 or HERCEPTIN® significantly inhibited the growth of the tumors. The com-

bination of HERCEPTIN®D and 2C4 or HERCEPTIN® and 7C2 was superior to either monoclonal antibody administered alone.

EXAMPLE 6

Treating Colorectal Cancer with Monoclonal Antibody 2C4 Human colorectal cell lines such as HCA-7, LS174T or CaCo-2 are implanted subcutaneously in athymic nude mice as described in Sheng et al. *J. Clin. Invest.* 99:2254–2259 (1997). Once tumors are established to about 100 mm^3 in volume, groups of animals are treated with 10–50 mg/kg of monoclonal antibody 2C4 administered twice weekly by injection in the intraperitoneal cavity. Monoclonal antibody 2C4 suppresses growth of colorecial xenografts in vivo.

EXAMPLE 7

Treating Breast Cancer with Humanized 2C4

The effect of rhuMAb 2C4 or HERCEPTIN® on human breast cancer cells which do not overexpress ErbB2 was assessed in a 3 day Alamar Blue assay (Ahmed, S. A. *J. Immunol. Methods* 170:211–224 (1994); and Page et al. *Int. J. Oncol.* 3:473–476(1994)). The cells used in this assay were MDA-175 human breast cancer cells which express ErbB2 at a 1+level. As shown in FIG. **12**, the growth of the breast cancer cell line, MDA-175, is significantly inhibited in a dose-dependent manner by the addition of rhuMAb 2C4 in comparison to HERCEPTIN® treatment.

The efficacy of rhuMAb 2C4 against MCF7 xenografts which are estrogen receptor positive (ER+) and express low levels of ErbB2 was assessed. Female mice supplemented with estrogen were used. rhuMAb 2C4 was administered at a dose of 30 mg/kg every week. As shown in FIG. **13**, rhuMAb 2C4 was effective in inhibiting breast cancer tumor growth in vivo, where the breast cancer was not characterized by overexpression of ErbB2.

EXAMPLE 8

Pharmacokinetics, Metabolism and Toxicology of 2C4

rhuMAb 2C4 was stable in human serum. No evidence or aggregates of complex formation in biological matrices was observed. In mice, rhuMAb2C4 cleared faster than HERCEPTIN®. Pharmacokinetic studies indicate that weekly administration of about 2–6 mg/kg of rhuMAb 2C4 should result in serum concentrations similar to HERCEPTIN®D as presently dosed. Resulting serum 2C4 exposure should greatly exceed IC, determined in vitro.

A toxicology study was carried out in cynomolgus monkeys (2 males and 2 females per group). rhuMab 2C4 was administered intravenously at 0, 10, 50 or 100 mg/kg twice a week for 4 weeks. The toxicology study measurements included body weights (−2, —1 weeks and weekly thereafter); food consumption (qualitative, daily); physical examinations with assessment of blood pressure, electrocardiogram (ECG), and body temperature (−2, −1 weeks and weeks 2 and 4, 4 hours post-dose following that weeks second dose); cardiac ultrasound evaluations (following first dose week 1 and end of study, week 4); clinical pathology (baseline and end of weeks 2 and 4); urinalysis (baseline and end of weeks 2 and 4); antibody analysis sampling (baseline and end of weeks 2 and 4); as well as necropsy and histopathology analysis.

All animals in all groups survived to the end of the study. No significant clinical observations, or differences among groups, were noted. Necropsy results showed no significant gross abnormalities in organs from any animals. No significant microscopic abnormalities were observed by in tissues from any of the animals. No significant changes in ECG were noted from initiation to completion of the study. In addition, no differences among the groups were seen.

EXAMPLE 9

Dose Escalation

Cancer patients are administered a first dose of rhuMAb 2C4 at one of five dose levels (0.05, 0.5, 2.0, 4.0 or 10 mg/kg; 6 subjects per dose level), followed by a 4 week wash-out. Week 5 patients are given the same dose weekly 4 times followed by a further 4 week wash-out. Patients with complete response, partial response or stable disease are eligible for extension studies.

EXAMPLE 10

Therapy of Relapsed or Refractory Metastatic Prostate Cancer

RhuMAb 2C4 is a full-length, humanized monoclonal antibody (produced in CHO cells) directed against ErbB2. RhuMab 2C4 blocks the associated of ErbB2 with other ErbB family members thereby inhibiting intracellular signaling through the ErbB pathway. In contrast to HERCEPTIN®, rhuMAb 2C4 not only inhibits the growth of ErbB2 overexpressing tumors but also blocks growth of tumors that require ErbB ligand-dependent signaling.

RhuMAb 2C4 is indicated as a single agent for treatment of hormone-refractory (androgen independent) prostate cancer patients. Primary endpoints for efficacy include overall survival compared to best available care (Mitoxantrone/Prednisone), when used as a single agent, and safety. Secondary efficacy endpoints include: time to disease progression, response rate, quality of life, pain and/or duration of response. RhuMAb 2C4 is administered intravenously (IV) weekly or every three weeks at 2 or 4 mg/kg, respectively, until disease progression. The antibody is supplied as a multi-dose liquid formulation (20 mL fill at a concentration of 20 mg/mL or higher concentration).

RhuMAb 2C4 is also indicated in combination with chemotherapy for treatment of hormone-refractory (androgen independent) prostate cancer patients. Primary endpoints for efficacy include overall survival compared to chemotherapy, and safety. Secondary efficacy endpoints include: time to disease progression, response rate, quality of life, pain and/or duration of response. RhuMAb 2C4 is administered intravenously (IV) weekly or every three weeks at 2 or 4 mg/kg, respectively, until disease progression. The antibody is supplied as a multi-dose liquid formulation (20 mL fill at a concentration of 20 mg/mL or higher concentration).

Examples of drugs that can be combined with the anti-ErbB2 antibody (which blocks ligand activation of an ErbB2 receptor) to treat prostate cancer (e.g. androgen independent prostate cancer) include a farnesyl transferase inhibitor; an anti-angiogenic agent (e.g. an anti-VEGF antibody); an EGFR-targeted drug (e.g. C225 or ZD1839); another anti-ErbB2 antibody (e.g. a growth inhibitory anti-ErbB2 antibody such as HERCEPTIN®, or an anti-ErbB2 antibody which induces apoptosis such as 7C2 or 7F3, including humanized and/or affinity matured variants thereof); a cytokine (e.g. IL-2, IL-12. G-CSF or GM-CSF); an anti-androgen (such as flutamide or cyproterone acetate); leu-

prolide; suramin; a chemotherapeutic agent such as vinblastine, estramustine, mitoxantrone, liarozole (a retinoic acid metabolism-blocking agent), cyclophosphamide, anthracycline antibiotics such as doxorubicin, a taxane (e.g. paclitaxel or docetaxel), or methotrexate, or any combination of the above, such as vinblastine/estramustine or cyclophosphamide/doxorubicin/methotrexate; prednisone; hydrocortizone; or combinations thereof. Standard doses for these various drugs can be administered, e.g. 40 mg/m^2/wk docetaxel (TAXOTERE®); 6 (AUC) carboplatin; and 200 mg/m^2 paclitaxel (TAXOL®).

EXAMPLE 11

Therapy of Metastatic Breast Cancer

RhuMAb 2C4 is indicated as a single agent for treatment of metastatic breast cancer patients whose tumors do not overexpress ErbB2. Primary endpoints for efficacy include response rate and safety. Secondary efficacy endpoints include: overall survival, time to disease progression, quality of life, and/or duration of response. RhuMAb 2C4 is administered intravenously (IV) weekly or every three weeks at 2 or 4 mg/kg, respectively, until disease progression. The antibody is supplied as a multi-dose liquid formulation (20 mL fill at a concentration of 20 mg/mL or higher concentration).

RhuMAb 2C4 is also indicated in combination with chemotherapy for treatment of metastatic breast cancer patients whose tumors do not overexpress ErbB2. Primary endpoints for efficacy include overall survival compared to chemotherapy alone, and safety. Secondary efficacy endpoints include: time to disease progression, response rate, quality of life, and/or duration of response. RhuMAb 2C4 is administered intravenously (IV) weekly or every three weeks at 2 or 4 mg/kg, respectively, until disease progression. The antibody is supplied as a multi-dose liquid formulation (20 mL fill at a concentration of 20 mg/mL or higher concentration).

Examples of drugs that can be combined with the anti-ErbB2 antibody (which blocks ligand activation of an ErbB2 receptor) to treat breast cancer (e.g. metastatic breast cancer which is not characterized by ErbB2 overexpression) include chemotherapeutic agents such as anthracycline antibiotics (e.g. doxorubicin), cyclophosphomide, a taxane (e.g. paclitaxel or docetaxel), navelbine, xeloda, mitomycin C, a platinum compound, oxaliplatin, gemcitabine, or combinations of two or more of these such as doxorubicin/cyclophosphomide; another anti-ErbB2 antibody (e.g. a growth inhibitory anti-ErbB2 antibody such as HERCEPTIN®, or an anti-ErbB2 antibody which induces apoptosis such as 7C2 or 7F3, including humanized or affinity matured variants thereof); an anti-estrogen (e.g. tamoxifen); a farnesyl transferase inhibitor; an anti-angiogenic agent (e.g. an anti-VEGF antibody); an EGFR-targeted drug (e.g. C225 or ZD1839); a cytokine (e.g. IL-2, IL-12, G-CSF or GM-CSF); or combinations of the above. Standard dosages for such additional drugs may be used.

RhuMAb 2C4 is additionally indicated in combination with HERCEPTIN® for treatment of metastatic breast cancer patients whose tumors overexpress ErbB2. Primary endpoints for efficacy include response rate, and safety. Secondary efficacy endpoints include: time to disease progression, overall survival compared to HERCEPTIN® alone, quality of life, and/or duration of response. RhuMAb 2C4 is administered intravenously (IV) weekly or every three weeks at 2 or 4 mg/kg, respectively, until disease progression. The antibody is supplied as a multi-dose liquid formulation (20 mL fill at a concentration of 20 mg/mL or higher concentration). HERCEPTIN® is administered IV as an initial loading dose of 4 mg/kg followed by a weekly maintenance dose of 2 mg/kg. HERCEPTIN® is supplied as a lyophilized powder. Each vial of HERCEPTIN® contains 440 mg HERCEPTIN®, 9.9 mg L-histidine HCl, 6.4 mg L-histidine, 400 mg α-α-trehalose dihydrate, and 1.8 mg polysorbate 20. Reconstitution with 20 mL of Bacteriostatic Water for Injection (BWFI) containing 1.1% benzyl alcohol as a preservative, yields 21 mL of a multi-dose solution containing 21 mg/mL HERCEPTIN®, at a pH of approximately 6.0.

EXAMPLE 12

Therapy of Lung Cancer

RhuMAb 2C4 is indicated as a single agent for treatment of stage IIIb or IV non-small cell lung cancer (NSCLC). Primary endpoints for efficacy include response rate, and safety. Secondary efficacy endpoints include: overall survival, time to disease progression, quality of life, and/or duration of response. RhuMAb 2C4 is administered intravenously (IV) weekly or every three weeks at 2 or 4 mg/kg, respectively, until disease progression. The antibody is supplied as a multi-dose liquid formulation (20 mL fill at a concentration of 20 mg/mL or higher concentration).

RhuMAb 2C4 is also indicated in combination with chemotherapy for treatment of metastatic non-small cell lung cancer patients. Primary endpoints for efficacy include overall survival compared to standard therapy, and safety. Secondary efficacy endpoints include: time to disease progression, response rate, quality of life and/or duration of response. RhuMAb 2C4 is administered intravenously (IV) weekly or every three weeks at 2 or 4 mg/kg, respectively, until disease progression. The antibody is supplied as a multi-dose liquid formulation (20 mL fill at a concentration of 20 mg/mL or higher concentration).

Examples of additional drugs which can be combined with the antibody (which binds ErbB2 and blocks ligand activation of an ErbB receptor) to treat lung cancer, include chemotherapeutic agents such as carboplatin, a taxane (e.g. paclitaxel or docetaxel), gemcitabine, navelbine, cisplatin, oxaliplatin, or combinations of any of these such as carboplatin/docetaxel; another anti-ErbB2 antibody (e.g. a growth inhibitory anti-ErbB2 antibody such as HERCEPTIN®, or an anti-ErbB2 antibody which induces apoptosis such as 7C2 or 7F3, including humanized or affinity matured variants thereof); a farnesyl transferase inhibitor; an anti-angiogenic agent (e.g. an anti-VEGF antibody); an EGFR-targeted drug (e.g. C225 or ZD1839); a cytokine (e.g. IL-2, IL-12, G-CSF or GM-CSF); or combinations of the above.

EXAMPLE 13

Therapy or Colorectal Cancer

RhuMAb 2C4 is indicated as a single agent for treatment of metastatic colorectal cancer. Primary endpoints for efficacy include response rate and safety. Secondary efficacy endpoints include: overall survival, time to disease progression, quality of life, and/or duration of response. RhuMAb 2C4 is administered intravenously (IV) weekly or every three weeks at 2 or 4 mg/kg, respectively, until disease progression. The antibody is supplied as a multi-dose liquid formulation (20 mL fill at a concentration of 20 mg/mL or higher concentration).

RhuMAb 2C4 is also indicated in combination with chemotherapy for treatment of metastatic colorectal cancer patients. Primary endpoints for efficacy include overall survival compared to standard therapy, and safety. Secondary efficacy endpoints include: time to disease progression, response rate, quality of life, and/or duration of response. RhuMAb 2C4 is administered intravenously (IV) weekly or every three weeks at 2 or 4 mg/kg, respectively, until disease progression. The antibody is supplied as a multi-dose liquid formulation (20 mL fill at a concentration of 20 mg/mL or higher concentration).

Examples of chemotherapeutic agents used to treat colorectal cancer, which can be combined with the antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor, include 5-fluorouracil (5-FU), leucovorin (LV), CPT-11, levamisole, or combinations of any two or more of these, e.g., 5-FU/LV/CPT-11. Standard dosages of such chemotherapeutic agents can be administered. Other drugs that may be combined with the anti-ErbB2 antibody to treat colorectal cancer include a farnesyl transferase inhibitor; an anti-angiogenic agent (e.g. an anti-VEGF antibody); an EGFR-targeted drug (e.g. C225 or ZD1839); a cytokine (e.g. IL-2, IL-12, G-CSF or GM-CSF); another anti-ErbB2 antibody (e.g. a growth inhibitory anti-ErbB2 antibody such as HERCEPTIN®, or an anti-ErbB2 antibody which induces apoptosis such as 7C2 or 7F3, including humanized or affinity matured variants thereof); or combinations of the above.

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35 40 45

Leu Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr Thr Gly Val Pro Ser
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Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
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Glu Trp Val Ala Asp Val Asn Pro Asn Ser Gly Gly Ser Ile Tyr
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Asn Gln Arg Phe Lys Gly Arg Phe Thr Leu Ser Val Asp Arg Ser
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Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
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Leu Leu Ile Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
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Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
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Glu Trp Val Ala Val Ile Ser Gly Asp Gly Gly Ser Thr Tyr Tyr
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Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser
65 70 75

Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
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<400> SEQUENCE: 11

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1 5

<210> SEQ ID NO 12
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 12

Gln Gln Tyr Tyr Ile Tyr Pro Tyr Thr
1 5

<210> SEQ ID NO 13
<211> LENGTH: 645
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu
1 5 10 15

Leu Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp
20 25 30

Met Lys Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met
35 40 45

Leu Arg His Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu
50 55 60

-continued

```
Glu Leu Thr Tyr Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln
                65                  70                  75

Asp Ile Gln Glu Val Gln Gly Tyr Val Leu Ile Ala His Asn Gln
                80                  85                  90

Val Arg Gln Val Pro Leu Gln Arg Leu Arg Ile Val Arg Gly Thr
                95                 100                 105

Gln Leu Phe Glu Asp Asn Tyr Ala Leu Ala Val Leu Asp Asn Gly
               110                 115                 120

Asp Pro Leu Asn Asn Thr Thr Pro Val Thr Gly Ala Ser Pro Gly
               125                 130                 135

Gly Leu Arg Glu Leu Gln Leu Arg Ser Leu Thr Glu Ile Leu Lys
               140                 145                 150

Gly Gly Val Leu Ile Gln Arg Asn Pro Gln Leu Cys Tyr Gln Asp
               155                 160                 165

Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn Asn Gln Leu Ala
               170                 175                 180

Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys His Pro Cys
               185                 190                 195

Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser Ser Glu
               200                 205                 210

Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys Ala
               215                 220                 225

Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
               230                 235                 240

Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys
               245                 250                 255

Leu His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala
               260                 265                 270

Leu Val Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro
               275                 280                 285

Glu Gly Arg Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro
               290                 295                 300

Tyr Asn Tyr Leu Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys
               305                 310                 315

Pro Leu His Asn Gln Glu Val Thr Ala Glu Asp Gly Thr Gln Arg
               320                 325                 330

Cys Glu Lys Cys Ser Lys Pro Cys Ala Arg Val Cys Tyr Gly Leu
               335                 340                 345

Gly Met Glu His Leu Arg Glu Val Arg Ala Val Thr Ser Ala Asn
               350                 355                 360

Ile Gln Glu Phe Ala Gly Cys Lys Lys Ile Phe Gly Ser Leu Ala
               365                 370                 375

Phe Leu Pro Glu Ser Phe Asp Gly Asp Pro Ala Ser Asn Thr Ala
               380                 385                 390

Pro Leu Gln Pro Glu Gln Leu Gln Val Phe Glu Thr Leu Glu Glu
               395                 400                 405

Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro Asp Ser Leu Pro
               410                 415                 420

Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg Gly Arg Ile
               425                 430                 435

Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu Gly Ile
               440                 445                 450
```

-continued

```
Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly Leu
                455                 460                 465

Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
                470                 475                 480

Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His
                485                 490                 495

Thr Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala
                500                 505                 510

Cys His Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro
                515                 520                 525

Thr Gln Cys Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys
                530                 535                 540

Val Glu Glu Cys Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val
                545                 550                 555

Asn Ala Arg His Cys Leu Pro Cys His Pro Glu Cys Gln Pro Gln
                560                 565                 570

Asn Gly Ser Val Thr Cys Phe Gly Pro Glu Ala Asp Gln Cys Val
                575                 580                 585

Ala Cys Ala His Tyr Lys Asp Pro Pro Phe Cys Val Ala Arg Cys
                590                 595                 600

Pro Ser Gly Val Lys Pro Asp Leu Ser Tyr Met Pro Ile Trp Lys
                605                 610                 615

Phe Pro Asp Glu Glu Gly Ala Cys Gln Pro Cys Pro Ile Asn Cys
                620                 625                 630

Thr His Ser Cys Val Asp Leu Asp Asp Lys Gly Cys Pro Ala Glu
                635                 640                 645
```

What is claimed is:

1. A method of treating cancer in a human, wherein the cancer expresses epidermal growth factor receptor (EGFR) and ErbB2, comprising administering to the human a therapeutically effective amount of an antibody which binds ErbB2 and blocks by 50% or greater binding of monoclonal antibody 2C4 (ATCC HB-12697) to ErbB2.

2. The method of claim 1 wherein the antibody blocks ligand activation of an ErbB receptor.

3. The method of claim 1 wherein the cancer is characterized by excessive activation of EGFR.

4. The method of claim 3 wherein the cancer overexpresses an ErbB ligand.

5. The method of claim, 5 wherein the ErbB ligand is transforming growth factor alpha (TGF-α).

6. The method of claim 1 wherein the antibody blocks TGF-α activation of mitogen-activated protein kinase (MAPK).

7. The method of claim 1 wherein the cancer is not characterized by overexpression of ErbB2 receptor.

8. The method of claim 1 wherein the cancer is selected from the group consisting of colon, rectal and colorectal cancer.

9. The method of claim 1 wherein the cancer is lung cancer.

10. The method of claim 9 wherein the cancer is non-small cell lung cancer.

11. The method of claim 1 wherein the antibody has a biological characteristic of monoclonal antibody 2C4 (ATCC HB-12697).

12. The method of claim 1 wherein the antibody is an antibody fragment.

13. The method of claim 12 wherein the antibody fragment is a Fab fragment.

14. The method of claim 1 wherein the antibody is not conjugated with a cytotoxic agent.

15. The method of claim 12 wherein the antibody fragment is not conjugated with a cytotoxic agent.

16. The method of claim 1 wherein the antibody is conjugated with a cytotoxic agent.

17. The method of claim 1 comprising administering at least one dose of the antibody to the human in an amount from about 0.5 mg/kg to about 10 mg/kg.

18. The method of claim 17 comprising administering the dose about every week.

19. The method of claim 17 comprising administering the dose about every three weeks.

20. A method of treating cancer in a human, wherein the cancer expresses but does not overexpress ErbB2 receptor, comprising administering to the human a therapeutically effective amount of an antibody which binds to ErbB2 and blocks ligand activation of an ErbB receptor more effectively than humanized monoclonal antibody huMAb4D5-8.

21. The method of claim 20 wherein the cancer is breast cancer.

22. The method of claim 21 wherein the cancer is metastatic breast cancer.

23. A method of treating cancer in a human, wherein the cancer is selected from the group consisting of colon, rectal and colorectal cancer which express but do not overexpress ErbB2, comprising administering to the human a therapeutically effective amount of an antibody which binds ErbB2 and blocks ligand activation of an ErbB receptor more effectively than humanized monoclonal antibody huMAb4D5-8.

24. A method of treating cancer in a human, wherein the cancer expresses epidermal growth factor receptor (EGFR) and ErbB2, comprising administering to the human a therapeutically effective amount of a humanized form of monoclonal antibody 2C4 (ATCC HB-12697).

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

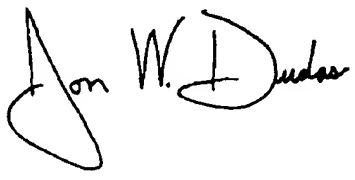
PATENT NO. : 6,949,245 B1
DATED : September 27, 2005
INVENTOR(S) : Mark Sliwkowski

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 75,
Line 50, please delete "claim, 5" and insert -- claim 4 --.

Signed and Sealed this

Sixth Day of December, 2005



JON W. DUDAS
Director of the United States Patent and Trademark Office

Attachment D

Certificate of Correction of U.S. Patent No. 6,949,245

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

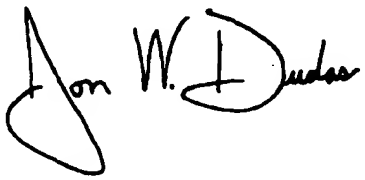
PATENT NO. : 6,949,245 B1
DATED : September 27, 2005
INVENTOR(S) : Mark Sliwkowski

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 75,
Line 50, please delete "claim, 5" and insert -- claim 4 --.

Signed and Sealed this

Sixth Day of December, 2005



JON W. DUDAS
Director of the United States Patent and Trademark Office

Attachment E

Evidence of Maintenance Fee Schedule for U.S. Patent No. 6,949,245

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

Customer No 000000000 | ISTMT | DATE PRINTED 07/29/2012

Genentech Inc
Attn Wendy Lee
1 DNA Way
San Francisco CA 94080-4990

MAINTENANCE FEE STATEMENT

According to the records of the U.S.Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O.Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,949,245	**$980.00**	**$0.00**	**02/25/09**	**09/602,812**	**09/27/05**	**06/23/00**	**04**	**NO**	**GENENTECH, INC.**

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United States Patent and Trademark Office

Patent Maintenance Fees		07/29/2012 09:54 PM EDT	
Patent Number:	6949245	**Application Number:**	09602812
Issue Date:	09/27/2005	**Filing Date:**	06/23/2000
Window Opens:	09/27/2012	**Surcharge Date:**	03/28/2013
Window Closes:	09/27/2013	**Payment Year:**	
Entity Status:	LARGE		
Customer Number:	0		
Address:	Genentech Inc Attn Wendy Lee 1 DNA Way San Francisco CA 94080-4990		
Phone Number:	(650) 225-1994		
Currently there are no fees due.			

Need Help? | USPTO Home Page | Finance Online Shopping Page | Alerts Page

Attachment F

Section of BLA providing pertuzumab's sequences

3.2.S.1.2 Structure [Pertuzumab, Genentech]

TABLE OF CONTENTS **Page**

3.2.S.1.2 Structure [Pertuzumab, Genentech]

Pertuzumab is a recombinant humanized monoclonal antibody based upon the human IgG1(κ) framework sequence composed of two light chains consisting of 214 amino acid residues and two heavy chains consisting of 448 or 449 amino acid residues. The sequences of the light and heavy chains of pertuzumab are shown in Figure 3.2.S.1.2–1 and Figure 3.2.S.1.2–2, respectively. Each light chain is covalently coupled through a disulfide link at cysteine 214 to a heavy-chain cysteine 222. The two heavy chains are covalently coupled to each other through two inter-chain disulfide bonds at residues cysteine 228 and cysteine 231 on each chain, consistent with the structure of a human IgG1.

The heavy chain of pertuzumab has a sequence of Ser-Pro-Gly-Lys at the C-terminus. It has been well documented that the C-terminal lysine in antibodies derived from mammalian cell cultures may be missing in the purified product, possibly as a result of post-translational processing by a host cell basic carboxypeptidease B (Harris 1995). The majority of heavy chain in pertuzumab has 448 amino acids because of lysine cleavage, as discussed in Section 3.2.S.3.1.

The C_H2 domain of each heavy chain also has a single conserved N-linked glycosylation site at Asn299, which is typical for IgG molecules. The biantennary neutral oligosaccharides present at this site are characteristic of the oligosaccharides observed in the Fc region of antibodies. Pertuzumab contains an Fc region identical to that of other humanized antibodies such as Herceptin® (trastuzumab), approved 25 September 1998 (STN: BL 103792).

REFERENCES

Harris RJ. Processing of C-terminal lysine and arginine residues of proteins isolated from mammalian cell culture. J Chromatogr A 1995;705:129–34.

Figure 3.2.S.1.2–1 Sequence of Pertuzumab Light Chain

```
1       10        20        30        40        50        60
|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
DIQMTQSPSSLSASVGDRVTITC**KASQDVSIGVA**WYQQKPGKAPKLLIY**SASYRYT**GVPS

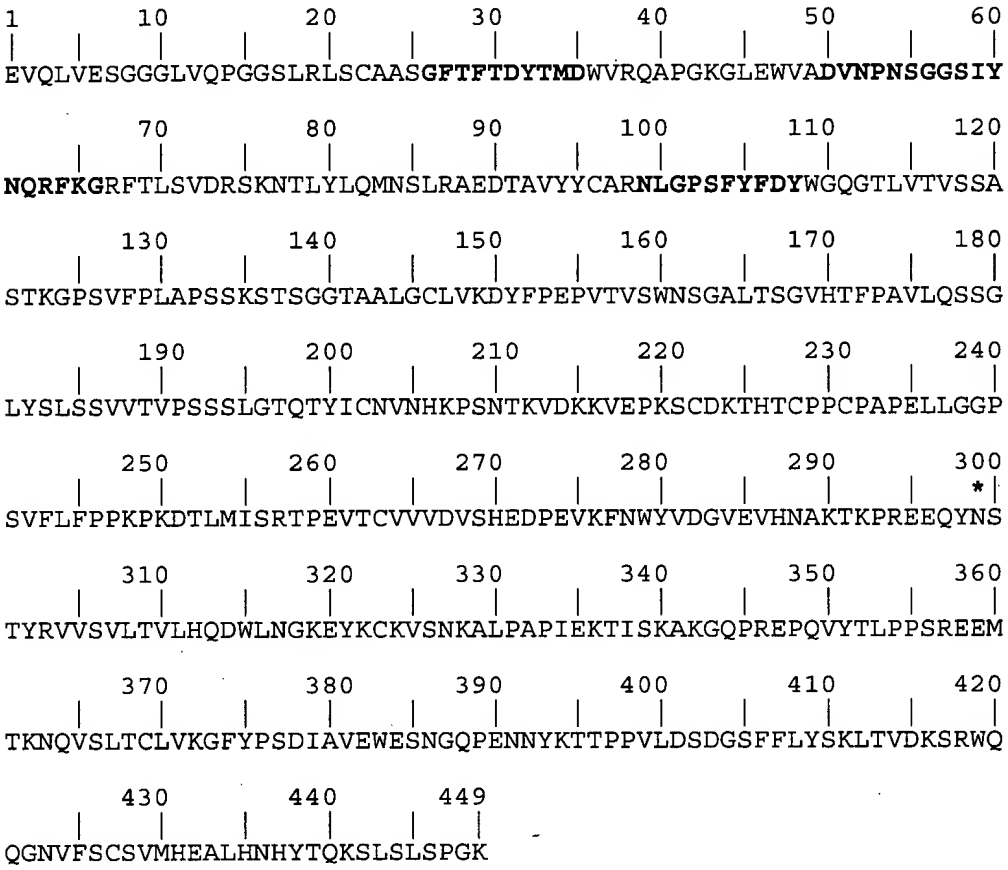
        70        80        90       100       110       120
    |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
RFSGSGSGTDFTLTISSLQPEDFATYYC**QQYYIYPYT**FGQGTKVEIKRTVAAPSVFIFPP

       130       140       150       160       170       180
    |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT

       190       200       210   214
    |   |   |   |   |   |   |   |
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
```

Complementarity-determining regions are shown in boldface type.
Calculated molecular mass is 23,526 Da (cysteine residues are in the reduced form).

Figure 3.2.S.1.2–2 Sequence of Pertuzumab Heavy Chain



Complementarity-determining regions are shown in boldface type.

* Marks the location of the carbohydrate moiety (Asn299).

Calculated molecular mass without carbohydrate is 49,217 Da (cysteine residues are in the reduced form).

Calculated molecular mass with neither lysine at the C-terminus or carbohydrate is 49,088 Da (cysteine residues are in the reduced form).

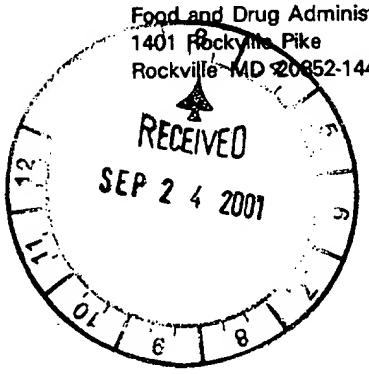
Attachment G

Letter from FDA to Genentech regarding IND acceptance/effective date

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

SEP 1 1 2001



Our Reference: BB-IND 9900

Genentech, Incorporated
Attention: Robert L. Garnick, Ph.D.
Senior Vice President
Quality, Regulatory Affairs, and Corporate Compliance
1 DNA Way
South San Francisco, CA 94080

27543

Dear Dr. Garnick:

We have reviewed your **Investigational New Drug Application (IND)** for "Humanized Monoclonal Antibody (rhuMAb 2C4) to HER2," and your study may proceed; however, we have the following comments, questions and requests for additional information:

1. Please submit the July 31 and August 6, 2001, facsimiles as amendments.

2. As product development continues, we strongly recommend removal of any intermediates that utilize human plasma-derived IgG or other material of animal or human origin from the manufacturing process.

3. Please submit the viral clearance validation study or a relevant cross-reference for the protein A chromatography process for rhuMAb CD11a (study no. V00-002-1049) that is cited in the process validation.

4. Please clarify the total production time from thaw to harvest during cell culture and fermentation and the product yield per volume from a single bulk harvest.

5. Please submit a stability protocol stating the timepoints at which rhuMAb 2C4 will be assayed following the different storage conditions.

6. Please clarify which equipment is dedicated to the purification process of rhuMAb 2C4, and which is not. Please clarify what validation procedures are in place to ensure avoiding cross-contamination with other products.

7. Please set a preliminary acceptance range for lot release for the potency assay.

8. As product development continues, you should provide additional information on the stability of the producer line. An extended fermentation test for genetic stability of the CHO cell transfectoma upon extended fermentation should be performed prior to studies beyond Phase 1 to detect potential plasmid stability problems. This fermentation should be performed at least once and should exceed the expected run time

by at least 50%. The cells at the end of this run should be tested for purity, identity, plasmid retention and integrity of the expression construct. We recommend that nucleotide analysis of the coding region for rhuMAb 2C4, including the flanking sequences, be performed at least once on the plasmid that is isolated from the end of production cells.

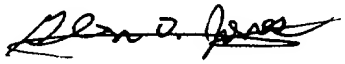
9. As product development continues, further testing or method validation should be performed for removal of tissue culture media supplements including methotrexate, insulin, and gentamycin.

10. Please submit the test procedure for the ELISA to be used for assessing serum levels of anti-rhuMAb 2C4 antibody. What are the sensitivity and specificity ranges of this assay?

11. Please submit the test procedure for the ELISA that will be used for quantifying rhuMAb 2C4 in patient serum and the sensitivity range of this assay.

If you have any questions, please contact the Regulatory Project Manager, Sharon Sickafuse, at (301) 827-5101.

Sincerely yours,



Glen D. Jones, Ph.D.
Director
Division of Application Review and Policy
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

Attachment H

Letter from the FDA to Genentech regarding receipt and acceptance of BLA Application

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

BL 125409/0

BLA ACKNOWLEDGEMENT
December 21, 2011

Genentech, Inc.
Attention: Josephine Ing
Sr. Scientist, Regulatory Affairs
1 DNA Way
South San Francisco, CA 94080-4990

Dear Ms. Ing:

We have received your Biologics License Application (BLA) submitted under section 351 of the Public Health Service Act (PHS Act) for the following:

Name of Biological Product: Pertuzumab for injection

Date of Application: December 6, 2011

Date of Receipt: December 8, 2011

Our Submission Tracking Number (STN): 125409/0

Proposed Use: For the treatment of patients with 1st Line HER2-positive metastatic breast cancer.

You are also responsible for complying with the applicable provisions of sections 402(i) and 402(j) of the Public Health Service Act (PHS Act) [42 USC §§ 282 (i) and (j)], which was amended by Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA) (Public Law No, 110-85, 121 Stat. 904).

Title VIII of FDAAA amended the PHS Act by adding new section 402(j) [42 USC § 282(j)], which expanded the current database known as ClinicalTrials.gov to include mandatory registration and reporting of results for applicable clinical trials of human drugs (including biological products) and devices.

In addition to the registration and reporting requirements described above, FDAAA requires that, at the time of submission of an application under section 505 of the FDCA, the application must be accompanied by a certification that all applicable requirements of 42 USC § 282(j) have been met. Where available, the certification must include the appropriate National Clinical Trial (NCT) numbers [42 USC § 282(j)(5)(B)].

You did not include such certification when you submitted this application, you may use Form FDA 3674, "Certification of Compliance, under 42 U.S.C. § 282(j)(5)(B), with Requirements of ClinicalTrials.gov Data Bank," [42 U.S.C. § 282(j)] to comply with the certification requirement. The form may be found at http://www.fda.gov/opacom/morechoices/fdaforms/default.html.

In completing Form FDA 3674, you should review 42 USC § 282(j) to determine whether the requirements of FDAAA apply to any clinical trial(s) referenced in this application. Please note that FDA published a guidance in January 2009, "Certifications To Accompany Drug, Biological Product, and Device Applications/Submissions: Compliance with Section 402(j) of The Public Health Service Act, Added By Title VIII of the Food and Drug Administration Amendments Act of 2007," that describes the Agency's current thinking regarding the types of applications and submissions that sponsors, industry, researchers, and investigators submit to the Agency and accompanying certifications. Additional information regarding the certification form is available at: http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/FoodandDrugAdministrationAmendmentsActof2007/ucm095442.htm. Additional information regarding Title VIII of FDAAA is available at: http://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-014.html. Additional information for registering your clinical trials is available at the Protocol Registration System website http://prsinfo.clinicaltrials.gov/.

When submitting the certification for this application, **do not** include the certification with other submissions to the application. Submit the certification within 30 days of the date of this letter. In the cover letter of the certification submission clearly identify that it pertains to **BLA 125409/0** submitted on December 6, 2011, and that it contains the FDA Form 3674 that was to accompany that application.

If you have already submitted the certification for this application, please disregard the above.

The BLA Submission Tracking Number provided above should be cited at the top of the first page of all submissions to this application. Send all submissions, electronic or paper, including those sent by overnight mail or courier, to the following address:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Oncology Products 1
5901-B Ammendale Road
Beltsville, MD 20705-1266

All regulatory documents submitted in paper should be three-hole punched on the left side of the page and bound. The left margin should be at least three-fourths of an inch to assure text is not obscured in the fastened area. Standard paper size (8-1/2 by 11 inches) should be used; however, it may occasionally be necessary to use individual pages larger than standard paper size. Non-standard, large pages should be folded and mounted to allow the page to be opened for review without disassembling the jacket and refolded without damage when the volume is shelved. Shipping unbound documents may result in the loss of portions of the submission or an

unnecessary delay in processing which could have an adverse impact on the review of the submission.

If you have any questions, call me at 301-796-3994.

Sincerely,

Amy R. Tilley

Amy R. Tilley
Regulatory Project Manager
Division of Oncology Products 1
Office of Hematology and Oncology Products
Center for Drug Evaluation and Research

Attachment I

Letter from the FDA to Genentech acknowledging receipt of IND 9900

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

JUL 0 9 2001

Our Reference: BB-IND 9900

Genentech, Incorporated
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Quality, Regulatory Affairs,
and Corporate Compliance
1 DNA Way
South San Francisco, CA 94080-4990

26905

Dear Dr. Garnick:

The Center for Biologics Evaluation and Research has received your **Investigational New Drug Application (IND)**. The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND #: 9900

SPONSOR: Genentech, Incorporated

PRODUCT NAME: Humanized Monoclonal Antibody (rhuMAb 2c4) to HER2

DATE OF SUBMISSION: June 29, 2001

DATE OF RECEIPT: July 2, 2001

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an **original and two copies of every submission to this file**. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file, you will be notified in writing of the reasons for placing the IND on hold.

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR Part 312, pertaining to INDs, is enclosed. Copies of other pertinent regulations are available from this Center upon request.

The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect [21 CFR 312.33]. Any unexpected, fatal or immediately life-threatening reaction associated with use of this product must be reported to this Division by telephone or facsimile transmission no later than seven calendar days after initial receipt of the information. All serious, unexpected adverse experiences, as well as results from animal studies that suggest significant clinical risk, must be reported, in writing, to this Division and to all investigators within fifteen calendar days after initial receipt of this information [21 CFR 312.32].

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 7a of form FDA 1571 requests that either an "environmental assessment," or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. See the enclosed information sheet for additional information on how these requirements may be addressed.

Telephone inquiries concerning this IND should be made directly to me at (301) 827-5101. Correspondence regarding this file should be addressed as follows:

Center for Biologics Evaluation and Research
Attn: Office of Therapeutics Research and Review
HFM-99, Room 200N
1401 Rockville Pike
Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Sharon Sickafuse

Sharon Sickafuse, M.S.
Regulatory Project Manager
Division of Application Review and Policy
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

Enclosures (3): 21 CFR Part 312
21 CFR 50.20, 50.25
Information sheet on 21 CFR 25.24

for marketing. In the absence of an approved new drug application or abbreviated new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use for the treatment and/or prevention of nocturnal leg muscle cramps is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After February 22, 1995, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[59 FR 43252, Aug. 22, 1994]

§ 310.547 Drug products containing quinine offered over-the-counter (OTC) for the treatment and/or prevention of malaria.

(a) Quinine and quinine salts have been used OTC for the treatment and/or prevention of malaria, a serious and potentially life-threatening disease. Quinine is no longer the drug of choice for the treatment and/or prevention of most types of malaria. In addition, there are serious and complicating aspects of the disease itself and some potentially serious and life-threatening risks associated with the use of quinine at doses employed for the treatment of malaria. There is a lack of adequate data to establish general recognition of the safety of quinine drug products for OTC use in the treatment and/or prevention of malaria. Therefore, quinine or quinine salts cannot be safely and effectively used for the treatment and/or prevention of malaria except under the care and supervision of a doctor.

(b) Any OTC drug product containing quinine or quinine salts that is labeled, represented, or promoted for the treatment and/or prevention of malaria is regarded as a new drug within the meaning of section 201(p) of the act, for which an approved application or abbreviated application under section 505 of the act and part 314 of this chapter is required for marketing. In the absence of an approved new drug application or abbreviated new drug application, such product is also misbranded under section 502 of the act.

(c) Clinical investigations designed to obtain evidence that any drug product labeled, represented, or promoted for OTC use for the treatment and/or prevention of malaria is safe and effective for the purpose intended must comply with the requirements and procedures governing the use of investigational new drugs set forth in part 312 of this chapter.

(d) After April 20, 1998, any such OTC drug product initially introduced or initially delivered for introduction into interstate commerce that is not in compliance with this section is subject to regulatory action.

[63 FR 13528, Mar. 20, 1998]

PART 312—INVESTIGATIONAL NEW DRUG APPLICATION

Subpart A—General Provisions

Subpart B—Investigational New Drug Application (IND)

Subpart C—Administrative Actions

Subpart D—Responsibilities of Sponsors and Investigators

Subpart E—Drugs Intended to Treat Life-threatening and Severely-debilitating Illnesses

Subpart F—Miscellaneous

Subpart G—Drugs for Investigational Use in Laboratory Research Animals or in Vitro Tests

AUTHORITY: 21 U.S.C. 321, 331, 351, 352, 353, 355, 371; 42 U.S.C. 262.

SOURCE: 52 FR 8831, Mar. 19, 1987, unless otherwise noted.

Subpart A—General Provisions

§ 312.1 Scope.

(a) This part contains procedures and requirements governing the use of investigational new drugs, including procedures and requirements for the submission to, and review by, the Food and Drug Administration of investigational new drug applications (IND's). An investigational new drug for which an IND is in effect in accordance with this part is exempt from the premarketing approval requirements that are otherwise applicable and may be shipped lawfully for the purpose of conducting clinical investigations of that drug.

(b) References in this part to regulations in the Code of Federal Regulations are to chapter I of title 21, unless otherwise noted.

§ 312.2 Applicability.

(a) *Applicability.* Except as provided in this section, this part applies to all clinical investigations of products that are subject to section 505 of the Federal Food, Drug, and Cosmetic Act or to the licensing provisions of the Public Health Service Act (58 Stat. 632, as amended (42 U.S.C. 201 *et seq.*)).

(b) *Exemptions.* (1) The clinical investigation of a drug product that is lawfully marketed in the United States is exempt from the requirements of this part if all the following apply:

(i) The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drug;

(ii) If the drug that is undergoing investigation is lawfully marketed as a

prescription drug product, the investigation is not intended to support a significant change in the advertising for the product;

(iii) The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product;

(iv) The investigation is conducted in compliance with the requirements for institutional review set forth in part 56 and with the requirements for informed consent set forth in part 50; and

(v) The investigation is conducted in compliance with the requirements of §312.7.

(2)(i) A clinical investigation involving an in vitro diagnostic biological product listed in paragraph (b)(2)(ii) of this section is exempt from the requirements of this part if (*a*) it is intended to be used in a diagnostic procedure that confirms the diagnosis made by another, medically established, diagnostic product or procedure and (*b*) it is shipped in compliance with §312.160.

(ii) In accordance with paragraph (b)(2)(i) of this section, the following products are exempt from the requirements of this part: (*a*) blood grouping serum; (*b*) reagent red blood cells; and (*c*) anti-human globulin.

(3) A drug intended solely for tests in vitro or in laboratory research animals is exempt from the requirements of this part if shipped in accordance with §312.160.

(4) FDA will not accept an application for an investigation that is exempt under the provisions of paragraph (b)(1) of this section.

(5) A clinical investigation involving use of a placebo is exempt from the requirements of this part if the investigation does not otherwise require submission of an IND.

(6) A clinical investigation involving an exception from informed consent under §50.24 of this chapter is not exempt from the requirements of this part.

(c) *Bioavailability studies.* The applicability of this part to in vivo bioavailability studies in humans is subject to the provisions of §320.31.

(d) *Unlabeled indication.* This part does not apply to the use in the practice of medicine for an unlabeled indication of a new drug product approved under part 314 or of a licensed biological product.

(e) *Guidance.* FDA may, on its own initiative, issue guidance on the applicability of this part to particular investigational uses of drugs. On request, FDA will advise on the applicability of this part to a planned clinical investigation.

[52 FR 8831, Mar. 19, 1987, as amended at 61 FR 51529, Oct. 2, 1996; 64 FR 401, Jan. 5, 1999]

EFFECTIVE DATE NOTE: At 64 FR 401, Jan. 5, 1999, §312.2 was amended by removing "or 507" from paragraph (a) and by removing "or antibiotic drug" from paragraph (d), effective May 20, 1999.

§312.3 Definitions and interpretations.

(a) The definitions and interpretations of terms contained in section 201 of the Act apply to those terms when used in this part:

(b) The following definitions of terms also apply to this part:

Act means the Federal Food, Drug, and Cosmetic Act (secs. 201-902, 52 Stat. 1040 *et seq.*, as amended (21 U.S.C. 301-392)).

Clinical investigation means any experiment in which a drug is administered or dispensed to, or used involving, one or more human subjects. For the purposes of this part, an experiment is any use of a drug except for the use of a marketed drug in the course of medical practice.

Contract research organization means a person that assumes, as an independent contractor with the sponsor, one or more of the obligations of a sponsor, e.g., design of a protocol, selection or monitoring of investigations, evaluation of reports, and preparation of materials to be submitted to the Food and Drug Administration.

FDA means the Food and Drug Administration.

IND means an investigational new drug application. For purposes of this part, "IND" is synonymous with "Notice of Claimed Investigational Exemption for a New Drug."

Investigational new drug means a new drug or biological drug that is used in a clinical investigation. The term also includes a biological product that is used in vitro for diagnostic purposes. The terms "investigational drug" and "investigational new drug" are deemed to be synonymous for purposes of this part.

Investigator means an individual who actually conducts a clinical investigation (i.e., under whose immediate direction the drug is administered or dispensed to a subject). In the event an investigation is conducted by a team of individuals, the investigator is the responsible leader of the team. "Subinvestigator" includes any other individual member of that team.

Marketing application means an application for a new drug submitted under section 505(b) of the Act or a product license application for a biological product submitted under the Public Health Service Act.

Sponsor means a person who takes responsibility for and initiates a clinical investigation. The sponsor may be an individual or pharmaceutical company, governmental agency, academic institution, private organization, or other organization. The sponsor does not actually conduct the investigation unless the sponsor is a sponsor-investigator. A person other than an individual that uses one or more of its own employees to conduct an investigation that it has initiated is a sponsor, not a sponsor-investigator, and the employees are investigators.

Sponsor-Investigator means an individual who both initiates and conducts an investigation, and under whose immediate direction the investigational drug is administered or dispensed. The term does not include any person other than an individual. The requirements applicable to a sponsor-investigator under this part include both those applicable to an investigator and a sponsor.

Subject means a human who participates in an investigation, either as a recipient of the investigational new drug or as a control. A subject may be a healthy human or a patient with a disease.

[52 FR 8831, Mar. 19, 1987, as amended at 64 FR 401, Jan. 5, 1999]

EFFECTIVE DATE NOTE: At 64 FR 401, Jan. 5, 1999, §312.3 was amended by removing ", antibiotic drug," from the paragraph defining "Investigational new drug" and by removing the phrase ", a request to provide for certification of an antibiotic submitted under section 507 of the Act," from the paragraph defining "Marketing application", effective May 20, 1999.

§312.6 Labeling of an investigational new drug.

(a) The immediate package of an investigational new drug intended for human use shall bear a label with the statement "Caution: New Drug—Limited by Federal (or United States) law to investigational use."

(b) The label or labeling of an investigational new drug shall not bear any statement that is false or misleading in any particular and shall not represent that the investigational new drug is safe or effective for the purposes for which it is being investigated.

§312.7 Promotion and charging for investigational drugs.

(a) *Promotion of an investigational new drug.* A sponsor or investigator, or any person acting on behalf of a sponsor or investigator, shall not represent in a promotional context that an investigational new drug is safe or effective for the purposes for which it is under investigation or otherwise promote the drug. This provision is not intended to restrict the full exchange of scientific information concerning the drug, including dissemination of scientific findings in scientific or lay media. Rather, its intent is to restrict promotional claims of safety or effectiveness of the drug for a use for which it is under investigation and to preclude commercialization of the drug before it is approved for commercial distribution.

(b) *Commercial distribution of an investigational new drug.* A sponsor or investigator shall not commercially distribute or test market an investigational new drug.

(c) *Prolonging an investigation.* A sponsor shall not unduly prolong an investigation after finding that the results of the investigation appear to establish sufficient data to support a marketing application.

(d) *Charging for and commercialization of investigational drugs*—(1) *Clinical trials under an IND.* Charging for an investigational drug in a clinical trial under an IND is not permitted without the prior written approval of FDA. In requesting such approval, the sponsor shall provide a full written explanation of why charging is necessary in order for the sponsor to undertake or continue the clinical trial, e.g., why distribution of the drug to test subjects should not be considered part of the normal cost of doing business.

(2) *Treatment protocol or treatment IND.* A sponsor or investigator may charge for an investigational drug for a treatment use under a treatment protocol or treatment IND provided: (i) There is adequate enrollment in the ongoing clinical investigations under the authorized IND; (ii) charging does not constitute commercial marketing of a new drug for which a marketing application has not been approved; (iii) the drug is not being commercially promoted or advertised; and (iv) the sponsor of the drug is actively pursuing marketing approval with due diligence. FDA must be notified in writing in advance of commencing any such charges, in an information amendment submitted under §312.31. Authorization for charging goes into effect automatically 30 days after receipt by FDA of the information amendment, unless the sponsor is notified to the contrary.

(3) *Noncommercialization of investigational drug.* Under this section, the sponsor may not commercialize an investigational drug by charging a price larger than that necessary to recover costs of manufacture, research, development, and handling of the investigational drug.

(4) *Withdrawal of authorization.* Authorization to charge for an investigational drug under this section may be withdrawn by FDA if the agency finds that the conditions underlying the authorization are no longer satisfied.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 19476, May 22, 1987]

§312.10 Waivers.

(a) A sponsor may request FDA to waive applicable requirement under this part. A waiver request may be submitted either in an IND or in an information amendment to an IND. In an emergency, a request may be made by telephone or other rapid communication means. A waiver request is required to contain at least one of the following:

(1) An explanation why the sponsor's compliance with the requirement is unnecessary or cannot be achieved;

(2) A description of an alternative submission or course of action that satisfies the purpose of the requirement; or

(3) Other information justifying a waiver.

(b) FDA may grant a waiver if it finds that the sponsor's noncompliance would not pose a significant and unreasonable risk to human subjects of the investigation and that one of the following is met:

(1) The sponsor's compliance with the requirement is unnecessary for the agency to evaluate the application, or compliance cannot be achieved;

(2) The sponsor's proposed alternative satisfies the requirement; or

(3) The applicant's submission otherwise justifies a waiver.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

Subpart B—Investigational New Drug Application (IND)

§312.20 Requirement for an IND.

(a) A sponsor shall submit an IND to FDA if the sponsor intends to conduct a clinical investigation with an investigational new drug that is subject to §312.2(a).

(b) A sponsor shall not begin a clinical investigation subject to §312.2(a) until the investigation is subject to an IND which is in effect in accordance with §312.40.

(c) A sponsor shall submit a separate IND for any clinical investigation involving an exception from informed consent under §50.24 of this chapter. Such a clinical investigation is not permitted to proceed without the prior written authorization from FDA. FDA shall provide a written determination 30 days after FDA receives the IND or earlier.

[52 FR 8831, Mar. 19, 1987, as amended at 61 FR 51529, Oct. 2, 1996; 62 FR 32479, June 16, 1997]

§312.21 Phases of an investigation.

An IND may be submitted for one or more phases of an investigation. The clinical investigation of a previously untested drug is generally divided into three phases. Although in general the phases are conducted sequentially, they may overlap. These three phases of an investigation are a follows:

(a) *Phase 1.* (1) Phase 1 includes the initial introduction of an investigational new drug into humans. Phase 1 studies are typically closely monitored and may be conducted in patients or normal volunteer subjects. These studies are designed to determine the metabolism and pharmacologic actions of the drug in humans, the side effects associated with increasing doses, and, if possible, to gain early evidence on effectiveness. During Phase 1, sufficient information about the drug's pharmacokinetics and pharmacological effects should be obtained to permit the design of well-controlled, scientifically valid, Phase 2 studies. The total number of subjects and patients included in Phase 1 studies varies with the drug, but is generally in the range of 20 to 80.

(2) Phase 1 studies also include studies of drug metabolism, structure-activity relationships, and mechanism of action in humans, as well as studies in which investigational drugs are used as research tools to explore biological phenomena or disease processes.

(b) *Phase 2.* Phase 2 includes the controlled clinical studies conducted to evaluate the effectiveness of the drug for a particular indication or indications in patients with the disease or condition under study and to determine the common short-term side effects and risks associated with the drug. Phase 2 studies are typically well controlled, closely monitored, and conducted in a relatively small number of patients, usually involving no more than several hundred subjects.

(c) *Phase 3.* Phase 3 studies are expanded controlled and uncontrolled trials. They are performed after preliminary evidence suggesting effectiveness of the drug has been obtained, and are intended to gather the additional information about effectiveness and safety that is needed to evaluate the overall benefit-risk relationship of the drug and to provide an adequate basis for physician labeling. Phase 3 studies usually include from several hundred to several thousand subjects.

§312.22 General principles of the IND submission.

(a) FDA's primary objectives in reviewing an IND are, in all phases of the investigation, to assure the safety and rights of subjects, and, in Phase 2 and 3, to help assure that the quality of the scientific evaluation of drugs is adequate to permit an evaluation of the drug's effectiveness and safety. Therefore, although FDA's review of Phase 1 submissions will focus on assessing the safety of Phase 1 investigations, FDA's review of Phases 2 and 3 submissions will also include an assessment of the scientific quality of the clinical investigations and the likelihood that the investigations will yield data capable of meeting statutory standards for marketing approval.

(b) The amount of information on a particular drug that must be submitted in an IND to assure the accomplishment of the objectives described in paragraph (a) of this section depends upon such factors as the novelty of the drug, the extent to which it has been studied previously, the known or suspected risks, and the developmental phase of the drug.

(c) The central focus of the initial IND submission should be on the general investigational plan and the protocols for specific human studies. Subsequent amendments to the IND that contain new or revised protocols should build logically on previous submissions and should be supported by additional information, including the results of animal toxicology studies or other human studies as appropriate. Annual reports to the IND should serve as the focus for reporting the status of studies being conducted under the IND and

should update the general investigational plan for the coming year.

(d) The IND format set forth in § 312.23 should be followed routinely by sponsors in the interest of fostering an efficient review of applications. Sponsors are expected to exercise considerable discretion, however, regarding the content of information submitted in each section, depending upon the kind of drug being studied and the nature of the available information. Section 312.23 outlines the information needed for a commercially sponsored IND for a new molecular entity. A sponsor-investigator who uses, as a research tool, an investigational new drug that is already subject to a manufacturer's IND or marketing application should follow the same general format, but ordinarily may, if authorized by the manufacturer, refer to the manufacturer's IND or marketing application in providing the technical information supporting the proposed clinical investigation. A sponsor-investigator who uses an investigational drug not subject to a manufacturer's IND or marketing application is ordinarily required to submit all technical information supporting the IND, unless such information may be referenced from the scientific literature.

§ 312.23 IND content and format.

(a) A sponsor who intends to conduct a clinical investigation subject to this part shall submit an "Investigational New Drug Application" (IND) including, in the following order:

(1) *Cover sheet (Form FDA-1571).* A cover sheet for the application containing the following:

(i) The name, address, and telephone number of the sponsor, the date of the application, and the name of the investigational new drug.

(ii) Identification of the phase or phases of the clinical investigation to be conducted.

(iii) A commitment not to begin clinical investigations until an IND covering the investigations is in effect.

(iv) A commitment that an Institutional Review Board (IRB) that complies with the requirements set forth in part 56 will be responsible for the initial and continuing review and approval of each of the studies in the proposed clinical investigation and the investigator will report to the IRB proposed changes in the research activity in accordance with the requirements of part 56.

(v) A commitment to conduct the investigation in accordance with all other applicable regulatory requirements.

(vi) The name and title of the person responsible for monitoring the conduct and progress of the clinical investigations.

(vii) The name(s) and title(s) of the person(s) responsible under § 312.32 for review and evaluation of information relevant to the safety of the drug.

(viii) If a sponsor has transferred any obligations for the conduct of any clinical study to a contract research organization, a statement containing the name and address of the contract research organization, identification of the clinical study, and a listing of the obligations transferred. If all obligations governing the conduct of the study have been transferred, a general statement of this transfer—in lieu of a listing of the specific obligations transferred—may be submitted.

(ix) The signature of the sponsor or the sponsor's authorized representative. If the person signing the application does not reside or have a place of business within the United States, the IND is required to contain the name and address of, and be countersigned by, an attorney, agent, or other authorized official who resides or maintains a place of business within the United States.

(2) *A table of contents.*

(3) *Introductory statement and general investigational plan.* (i) A brief introductory statement giving the name of the drug and all active ingredients, the drug's pharmacological class, the structural formula of the drug (if known), the formulation of the dosage form(s) to be used, the route of administration, and the broad objectives and planned duration of the proposed clinical investigation(s).

(ii) A brief summary of previous human experience with the drug, with reference to other IND's if pertinent, and to investigational or marketing experience in other countries that may be relevant to the safety of the proposed clinical investigation(s).

(iii) If the drug has been withdrawn from investigation or marketing in any country for any reason related to safety or effectiveness, identification of the country(ies) where the drug was withdrawn and the reasons for the withdrawal.

(iv) A brief description of the overall plan for investigating the drug product for the following year. The plan should include the following: (*a*) The rationale for the drug or the research study; (*b*) the indication(s) to be studied; (*c*) the general approach to be followed in evaluating the drug; (*d*) the kinds of clinical trials to be conducted in the first year following the submission (if plans are not developed for the entire year, the sponsor should so indicate); (*e*) the estimated number of patients to be given the drug in those studies; and (*f*) any risks of particular severity or seriousness anticipated on the basis of the toxicological data in animals or prior studies in humans with the drug or related drugs.

(4) [Reserved]

(5) *Investigator's brochure.* If required under § 312.55, a copy of the investigator's brochure, containing the following information:

(i) A brief description of the drug substance and the formulation, including the structural formula, if known.

(ii) A summary of the pharmacological and toxicological effects of the drug in animals and, to the extent known, in humans.

(iii) A summary of the pharmacokinetics and biological disposition of the drug in animals and, if known, in humans.

(iv) A summary of information relating to safety and effectiveness in humans obtained from prior clinical studies. (Reprints of published articles on such studies may be appended when useful.)

(v) A description of possible risks and side effects to be anticipated on the basis of prior experience with the drug under investigation or with related drugs, and of precautions or special monitoring to be done as part of the investigational use of the drug.

(6) *Protocols.* (i) A protocol for each planned study. (Protocols for studies not submitted initially in the IND should be submitted in accordance with § 312.30(a).) In general, protocols for Phase 1 studies may be less detailed and more flexible than protocols for Phase 2 and 3 studies. Phase 1 protocols should be directed primarily at providing an outline of the investigation—an estimate of the number of patients to be involved, a description of safety exclusions, and a description of the dosing plan including duration, dose, or method to be used in determining dose—and should specify in detail only those elements of the study that are critical to safety, such as necessary monitoring of vital signs and blood chemistries. Modifications of the experimental design of Phase 1 studies that do not affect critical safety assessments are required to be reported to FDA only in the annual report.

(ii) In Phases 2 and 3, detailed protocols describing all aspects of the study should be submitted. A protocol for a Phase 2 or 3 investigation should be designed in such a way that, if the sponsor anticipates that some deviation from the study design may become necessary as the investigation progresses, alternatives or contingencies to provide for such deviation are built into the protocols at the outset. For example, a protocol for a controlled short-term study might include a plan for an early crossover of nonresponders to an alternative therapy.

(iii) A protocol is required to contain the following, with the specific elements and detail of the protocol reflecting the above distinctions depending on the phase of study:

(*a*) A statement of the objectives and purpose of the study.

(*b*) The name and address and a statement of the qualifications (curriculum vitae or other statement of qualifications) of each investigator, and the name of each subinvestigator (e.g., research fellow, resident) working under the supervision of the investigator; the name and address of the research facilities to be used; and the name and address of each reviewing Institutional Review Board.

(*c*) The criteria for patient selection and for exclusion of patients and an estimate of the number of patients to be studied.

(d) A description of the design of the study, including the kind of control group to be used, if any, and a description of methods to be used to minimize bias on the part of subjects, investigators, and analysts.

(e) The method for determining the dose(s) to be administered, the planned maximum dosage, and the duration of individual patient exposure to the drug.

(f) A description of the observations and measurements to be made to fulfill the objectives of the study.

(g) A description of clinical procedures, laboratory tests, or other measures to be taken to monitor the effects of the drug in human subjects and to minimize risk.

(7) *Chemistry, manufacturing, and control information.* (i) As appropriate for the particular investigations covered by the IND, a section describing the composition, manufacture, and control of the drug substance and the drug product. Although in each phase of the investigation sufficient information is required to be submitted to assure the proper identification, quality, purity, and strength of the investigational drug, the amount of information needed to make that assurance will vary with the phase of the investigation, the proposed duration of the investigation, the dosage form, and the amount of information otherwise available. FDA recognizes that modifications to the method of preparation of the new drug substance and dosage form and changes in the dosage form itself are likely as the investigation progresses. Therefore, the emphasis in an initial Phase 1 submission should generally be placed on the identification and control of the raw materials and the new drug substance. Final specifications for the drug substance and drug product are not expected until the end of the investigational process.

(ii) It should be emphasized that the amount of information to be submitted depends upon the scope of the proposed clinical investigation. For example, although stability data are required in all phases of the IND to demonstrate that the new drug substance and drug product are within acceptable chemical and physical limits for the planned duration of the proposed clinical investigation, if very short-term tests are proposed, the supporting stability data can be correspondingly limited.

(iii) As drug development proceeds and as the scale or production is changed from the pilot-scale production appropriate for the limited initial clinical investigations to the larger-scale production needed for expanded clinical trials, the sponsor should submit information amendments to supplement the initial information submitted on the chemistry, manufacturing, and control processes with information appropriate to the expanded scope of the investigation.

(iv) Reflecting the distinctions described in this paragraph (a)(7), and based on the phase(s) to be studied, the submission is required to contain the following:

(*a*) *Drug substance.* A description of the drug substance, including its physical, chemical, or biological characteristics; the name and address of its manufacturer; the general method of preparation of the drug substance; the acceptable limits and analytical methods used to assure the identity, strength, quality, and purity of the drug substance; and information sufficient to support stability of the drug substance during the toxicological studies and the planned clinical studies. Reference to the current edition of the United States Pharmacopeia—National Formulary may satisfy relevant requirements in this paragraph.

(*b*) *Drug product.* A list of all components, which may include reasonable alternatives for inactive compounds, used in the manufacture of the investigational drug product, including both those components intended to appear in the drug product and those which may not appear but which are used in the manufacturing process, and, where applicable, the quantitative composition of the investigational drug product, including any reasonable variations that may be expected during the investigational stage; the name and address of the drug product manufacturer; a brief general description of the manufacturing and packaging procedure as appropriate for the product; the acceptable limits and analytical methods used to assure the identity, strength, quality, and purity of the drug product; and information sufficient to assure the product's stability during the planned clinical studies. Reference to the current edition of the United States Pharmacopeia—National Formulary may satisfy certain requirements in this paragraph.

(*c*) A brief general description of the composition, manufacture, and control of any placebo used in a controlled clinical trial.

(*d*) *Labeling.* A copy of all labels and labeling to be provided to each investigator.

(*e*) *Environmental analysis requirements.* A claim for categorical exclusion under § 25.30 or 25.31 or an environmental assessment under § 25.40.

(8) *Pharmacology and toxicology information.* Adequate information about pharmacological and toxicological studies of the drug involving laboratory animals or in vitro, on the basis of which the sponsor has concluded that it is reasonably safe to conduct the proposed clinical investigations. The kind, duration, and scope of animal and other tests required varies with the duration and nature of the proposed clinical investigations. Guidelines are available from FDA that describe ways in which these requirements may be met. Such information is required to include the identification and qualifications of the individuals who evaluated the results of such studies and concluded that it is reasonably safe to begin the proposed investigations and a statement of where the investigations were conducted and where the records are available for inspection. As drug development proceeds, the sponsor is required to submit informational amendments, as appropriate, with additional information pertinent to safety.

(i) *Pharmacology and drug disposition.* A section describing the pharmacological effects and mechanism(s) of action of the drug in animals, and information on the absorption, distribution, metabolism, and excretion of the drug, if known.

(ii) *Toxicology.* (*a*) An integrated summary of the toxicological effects of the drug in animals and in vitro. Depending on the nature of the drug and the phase of the investigation, the description is to include the results of acute, subacute, and chronic toxicity tests; tests of the drug's effects on reproduction and the developing fetus; any special toxicity test related to the drug's particular mode of administration or conditions of use (e.g., inhalation, dermal, or ocular toxicology); and any in vitro studies intended to evaluate drug toxicity.

(*b*) For each toxicology study that is intended primarily to support the safety of the proposed clinical investigation, a full tabulation of data suitable for detailed review.

(iii) For each nonclinical laboratory study subject to the good laboratory practice regulations under part 58, a statement that the study was conducted in compliance with the good laboratory practice regulations in part 58, or, if the study was not conducted in compliance with those regulations, a brief statement of the reason for the noncompliance.

(9) *Previous human experience with the investigational drug.* A summary of previous human experience known to the applicant, if any, with the investigational drug. The information is required to include the following:

(i) If the investigational drug has been investigated or marketed previously, either in the United States or other countries, detailed information about such experience that is relevant to the safety of the proposed investigation or to the investigation's rationale. If the durg has been the subject of controlled trials, detailed information on such trials that is relevant to an assessment of the drug's effectiveness for the proposed investigational use(s) should also be provided. Any published material that is relevant to the safety of the proposed investigation or to an assessment of the drug's effectiveness for its proposed investigational use should be provided in full. Published material that is less directly relevant may be supplied by a bibliography.

(ii) If the drug is a combination of drugs previously investigated or marketed, the information required under paragraph (a)(9)(i) of this section should be provided for each active drug component. However, if any component in such combination is subject to an approved marketing application or is otherwise lawfully marketed in the

United States, the sponsor is not required to submit published material concerning that active drug component unless such material relates directly to the proposed investigational use (including publications relevant to component-component interaction).

(iii) If the drug has been marketed outside the United States, a list of the countries in which the drug has been marketed and a list of the countries in which the drug has been withdrawn from marketing for reasons potentially related to safety or effectiveness.

(10) *Additional information.* In certain applications, as described below, information on special topics may be needed. Such information shall be submitted in this section as follows:

(i) *Drug dependence and abuse potential.* If the drug is a psychotropic substance or otherwise has abuse potential, a section describing relevant clinical studies and experience and studies in test animals.

(ii) *Radioactive drugs.* If the drug is a radioactive drug, sufficient data from animal or human studies to allow a reasonable calculation of radiation-absorbed dose to the whole body and critical organs upon administration to a human subject. Phase 1 studies of radioactive drugs must include studies which will obtain sufficient data for dosimetry calculations.

(iii) *Pediatric studies.* Plans for assessing pediatric safety and effectiveness.

(iv) *Other information.* A brief statement of any other information that would aid evaluation of the proposed clinical investigations with respect to their safety or their design and potential as controlled clinical trials to support marketing of the drug.

(11) *Relevant information.* If requested by FDA, any other relevant information needed for review of the application.

(b) *Information previously submitted.* The sponsor ordinarily is not required to resubmit information previously submitted, but may incorporate the information by reference. A reference to information submitted previously must identify the file by name, reference number, volume, and page number where the information can be found. A reference to information submitted to the agency by a person other than the sponsor is required to contain a written statement that authorizes the reference and that is signed by the person who submitted the information.

(c) *Material in a foreign language.* The sponsor shall submit an accurate and complete English translation of each part of the IND that is not in English. The sponsor shall also submit a copy of each original literature publication for which an English translation is submitted.

(d) *Number of copies.* The sponsor shall submit an original and two copies of all submissions to the IND file, including the original submission and all amendments and reports.

(e) *Numbering of IND submissions.* Each submission relating to an IND is required to be numbered serially using a single, three-digit serial number. The initial IND is required to be numbered 000; each subsequent submission (e.g., amendment, report, or correspondence) is required to be numbered chronologically in sequence.

(f) *Identification of exception from informed consent.* If the investigation involves an exception from informed consent under §50.24 of this chapter, the sponsor shall prominently identify on the cover sheet that the investigation is subject to the requirements in §50.24 of this chapter.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 53 FR 1918, Jan. 25, 1988; 61 FR 51529, Oct. 2, 1996; 62 FR 10599, July 29, 1997; 63 FR 66669, Dec. 2, 1998]

§312.30 Protocol amendments.

Once an IND is in effect, a sponsor shall amend it as needed to ensure that the clinical investigations are conducted according to protocols included in the application. This section sets forth the provisions under which new protocols may be submitted and changes in previously submitted protocols may be made. Whenever a sponsor intends to conduct a clinical investigation with an exception from informed consent for emergency research as set forth in §50.24 of this chapter, the sponsor shall submit a separate IND for such investigation.

(a) *New protocol.* Whenever a sponsor intends to conduct a study that is not covered by a protocol already contained in the IND, the sponsor shall submit to FDA a protocol amendment containing the protocol for the study. Such study may begin provided two conditions are met: (1) The sponsor has submitted the protocol to FDA for its review; and (2) the protocol has been approved by the Institutional Review Board (IRB) with responsibility for review and approval of the study in accordance with the requirements of part 56. The sponsor may comply with these two conditions in either order.

(b) *Changes in a protocol.* (1) A sponsor shall submit a protocol amendment describing any change in a Phase 1 protocol that significantly affects the safety of subjects or any change in a Phase 2 or 3 protocol that significantly affects the safety of subjects, the scope of the investigation, or the scientific quality of the study. Examples of changes requiring an amendment under this paragraph include:

(i) Any increase in drug dosage or duration of exposure of individual subjects to the drug beyond that in the current protocol, or any significant increase in the number of subjects under study.

(ii) Any significant change in the design of a protocol (such as the addition or dropping of a control group).

(iii) The addition of a new test or procedure that is intended to improve monitoring for, or reduce the risk of, a side effect or adverse event; or the dropping of a test intended to monitor safety.

(2)(i) A protocol change under paragraph (b)(1) of this section may be made provided two conditions are met:

(a) The sponsor has submitted the change to FDA for its review; and

(b) The change has been approved by the IRB with responsibility for review and approval of the study. The sponsor may comply with these two conditions in either order.

(ii) Notwithstanding paragraph (b)(2)(i) of this section, a protocol change intended to eliminate an apparent immediate hazard to subjects may be implemented immediately provided FDA is subsequently notified by protocol amendment and the reviewing IRB is notified in accordance with §56.104(c).

(c) *New investigator.* A sponsor shall submit a protocol amendment when a new investigator is added to carry out a previously submitted protocol, except that a protocol amendment is not required when a licensed practitioner is added in the case of a treatment protocol under §312.34. Once the investigator is added to the study, the investigational drug may be shipped to the investigator and the investigator may begin participating in the study. The sponsor shall notify FDA of the new investigator within 30 days of the investigator being added.

(d) *Content and format.* A protocol amendment is required to be prominently identified as such (i.e., "Protocol Amendment: New Protocol", "Protocol Amendment: Change in Protocol", or "Protocol Amendment: New Investigator"), and to contain the following:

(1)(i) In the case of a new protocol, a copy of the new protocol and a brief description of the most clinically significant differences between it and previous protocols.

(ii) In the case of a change in protocol, a brief description of the change and reference (date and number) to the submission that contained the protocol.

(iii) In the case of a new investigator, the investigator's name, the qualifications to conduct the investigation, reference to the previously submitted protocol, and all additional information about the investigator's study as is required under §312.23(a)(6)(iii)(b).

(2) Reference, if necessary, to specific technical information in the IND or in a concurrently submitted information amendment to the IND that the sponsor relies on to support any clinically significant change in the new or amended protocol. If the reference is made to supporting information already in the IND, the sponsor shall identify by name, reference number, volume, and page number the location of the information.

(3) If the sponsor desires FDA to comment on the submission, a request for such comment and the specific questions FDA's response should address.

(e) *When submitted.* A sponsor shall submit a protocol amendment for a new protocol or a change in protocol before its implementation. Protocol amendments to add a new investigator or to provide additional information about investigators may be grouped and submitted at 30-day intervals. When several submissions of new protocols or protocol changes are anticipated during a short period, the sponsor is encouraged, to the extent feasible, to include these all in a single submission.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 53 FR 1918, Jan. 25, 1988; 61 FR 51530, Oct. 2, 1996]

§ 312.31 Information amendments.

(a) *Requirement for information amendment.* A sponsor shall report in an information amendment essential information on the IND that is not within the scope of a protocol amendment, IND safety reports, or annual report. Examples of information requiring an information amendment include:

(1) New toxicology, chemistry, or other technical information; or

(2) A report regarding the discontinuance of a clinical investigation.

(b) *Content and format of an information amendment.* An information amendment is required to bear prominent identification of its contents (e.g., "Information Amendment: Chemistry, Manufacturing, and Control", "Information Amendment: Pharmacology-Toxicology", "Information Amendment: Clinical"), and to contain the following:

(1) A statement of the nature and purpose of the amendment.

(2) An organized submission of the data in a format appropriate for scientific review.

(3) If the sponsor desires FDA to comment on an information amendment, a request for such comment.

(c) *When submitted.* Information amendments to the IND should be submitted as necessary but, to the extent feasible, not more than every 30 days.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 53 FR 1918, Jan. 25, 1988]

§ 312.32 IND safety reports.

(a) *Definitions.* The following definitions of terms apply to this section:

Associated with the use of the drug. There is a reasonable possibility that the experience may have been caused by the drug.

Disability. A substantial disruption of a person's ability to conduct normal life functions.

Life-threatening adverse drug experience. Any adverse drug experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse drug experience: Any adverse drug experience occurring at any dose that results in any of the following outcomes: Death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Unexpected adverse drug experience: Any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure only referred to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure only listed cerebral vascular accidents. "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the investigator brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

(b) *Review of safety information.* The sponsor shall promptly review all information relevant to the safety of the drug obtained or otherwise received by the sponsor from any source, foreign or domestic, including information derived from any clinical or epidemiological investigations, animal investigations, commercial marketing experience, reports in the scientific literature, and unpublished scientific papers, as well as reports from foreign regulatory authorities that have not already been previously reported to the agency by the sponsor.

(c) *IND safety reports.* (1) *Written reports.*—(i) The sponsor shall notify FDA and all participating investigators in a written IND safety report of:

(A) Any adverse experience associated with the use of the drug that is both serious and unexpected; or

(B) Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity. Each notification shall be made as soon as possible and in no event later than 15 calendar days after the sponsor's initial receipt of the information. Each written notification may be submitted on FDA Form 3500A or in a narrative format (foreign events may be submitted either on an FDA Form 3500A or, if preferred, on a CIOMS I form; reports from animal or epidemiological studies shall be submitted in a narrative format) and shall bear prominent identification of its contents, i.e., "IND Safety Report." Each written notification to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND. If FDA determines that additional data are needed, the agency may require further data to be submitted.

(ii) In each written IND safety report, the sponsor shall identify all safety reports previously filed with the IND concerning a similar adverse experience, and shall analyze the significance of the adverse experience in light of the previouos, similar reports.

(2) *Telephone and facsimile transmission safety reports.* The sponsor shall also notify FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but in no event later than 7 calendar days after the sponsor's initial receipt of the information. Each telephone call or facsimile transmission to FDA shall be transmitted to the FDA new drug review division in the Center for Drug Evaluation and Research or the product review division in the Center for Biologics Evaluation and Research that has responsibility for review of the IND.

(3) *Reporting format or frequency.* FDA may request a sponsor to submit IND safety reports in a format or at a frequency different than that required under this paragraph. The sponsor may also propose and adopt a different reporting format or frequency if the change is agreed to in advance by the director of the new drug review division in the Center for Drug Evaluation and Research or the director of the products review division in the Center for Biologics Evaluation and Research which is responsible for review of the IND.

(4) A sponsor of a clinical study of a marketed drug is not required

a safety report for any adverse experience associated with use of the drug that is not from the clinical study itself.

(d) *Followup.* (1) The sponsor shall promptly investigate all safety information received by it.

(2) Followup information to a safety report shall be submitted as soon as the relevant information is available.

(3) If the results of a sponsor's investigation show that an adverse drug experience not initially determined to be reportable under paragraph (c) of this section is so reportable, the sponsor shall report such experience in a written safety report as soon as possible, but in no event later than 15 calendar days after the determination is made.

(4) Results of a sponsor's investigation of other safety information shall be submitted, as appropriate, in an information amendment or annual report.

(e) *Disclaimer.* A safety report or other information submitted by a sponsor under this part (and any release by FDA of that report or information) does not necessarily reflect a conclusion by the sponsor or FDA that the report or information constitutes an admission that the drug caused or contributed to an adverse experience. A sponsor need not admit, and may deny, that the report or information submitted by the sponsor constitutes an admission that the drug caused or contributed to an adverse experience.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11579, Mar. 29, 1990; 62 FR 52250, Oct. 7, 1997]

§ 312.33 Annual reports.

A sponsor shall within 60 days of the anniversary date that the IND went into effect, submit a brief report of the progress of the investigation that includes:

(a) *Individual study information.* A brief summary of the status of each study in progress and each study completed during the previous year. The summary is required to include the following information for each study:

(1) The title of the study (with any appropriate study identifiers such as protocol number), its purpose, a brief statement identifying the patient population, and a statement as to whether the study is completed.

(2) The total number of subjects initially planned for inclusion in the study; the number entered into the study to date, tabulated by age group, gender, and race; the number whose participation in the study was completed as planned; and the number who dropped out of the study for any reason.

(3) If the study has been completed, or if interim results are known, a brief description of any available study results.

(b) *Summary information.* Information obtained during the previous year's clinical and nonclinical investigations, including:

(1) A narrative or tabular summary showing the most frequent and most serious adverse experiences by body system.

(2) A summary of all IND safety reports submitted during the past year.

(3) A list of subjects who died during participation in the investigation, with the cause of death for each subject.

(4) A list of subjects who dropped out during the course of the investigation in association with any adverse experience, whether or not thought to be drug related.

(5) A brief description of what, if anything, was obtained that is pertinent to an understanding of the drug's actions, including, for example, information about dose response, information from controlled trails, and information about bioavailability.

(6) A list of the preclinical studies (including animal studies) completed or in progress during the past year and a summary of the major preclinical findings.

(7) A summary of any significant manufacturing or microbiological changes made during the past year.

(c) A description of the general investigational plan for the coming year to replace that submitted 1 year earlier. The general investigational plan shall contain the information required under § 312.23(a)(3)(iv).

(d) If the investigator brochure has been revised, a description of the revision and a copy of the new brochure.

(e) A description of any significant Phase 1 protocol modifications made during the previous year and not previously reported to the IND in a protocol amendment.

(f) A brief summary of significant foreign marketing developments with the drug during the past year, such as approval of marketing in any country or withdrawal or suspension from marketing in any country.

(g) If desired by the sponsor, a log of any outstanding business with respect to the IND for which the sponsor requests or expects a reply, comment, or meeting.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 63 FR 6862, Feb. 11, 1998]

§ 312.34 Treatment use of an investigational new drug.

(a) *General.* A drug that is not approved for marketing may be under clinical investigation for a serious or immediately life-threatening disease condition in patients for whom no comparable or satisfactory alternative drug or other therapy is available. During the clinical investigation of the drug, it may be appropriate to use the drug in the treatment of patients not in the clinical trials, in accordance with a treatment protocol or treatment IND. The purpose of this section is to facilitate the availability of promising new drugs to desperately ill patients as early in the drug development process as possible, before general marketing begins, and to obtain additional data on the drug's safety and effectiveness. In the case of a serious disease, a drug ordinarily may be made available for treatment use under this section during Phase 3 investigations or after all clinical trials have been completed; however, in appropriate circumstances, a drug may be made available for treatment use during Phase 2. In the case of an immediately life-threatening disease, a drug may be made available for treatment use under this section earlier than Phase 3, but ordinarily not earlier than Phase 2. For purposes of this section, the "treatment use" of a drug includes the use of a drug for diagnostic purposes. If a protocol for an investigational drug meets the criteria of this section, the protocol is to be submitted as a treatment protocol under the provisions of this section.

(b) *Criteria.* (1) FDA shall permit an investigational drug to be used for a treatment use under a treatment protocol or treatment IND if:

(i) The drug is intended to treat a serious or immediately life-threatening disease;

(ii) There is no comparable or satisfactory alternative drug or other therapy available to treat that stage of the disease in the intended patient population;

(iii) The drug is under investigation in a controlled clinical trial under an IND in effect for the trial, or all clinical trials have been completed; and

(iv) The sponsor of the controlled clinical trial is actively pursuing marketing approval of the investigational drug with due diligence.

(2) *Serious disease.* For a drug intended to treat a serious disease, the Commissioner may deny a request for treatment use under a treatment protocol or treatment IND if there is insufficient evidence of safety and effectiveness to support such use.

(3) *Immediately life-threatening disease.* (i) For a drug intended to treat an immediately life-threatening disease, the Commissioner may deny a request for treatment use of an investigational drug under a treatment protocol or treatment IND if the available scientific evidence, taken as a whole, fails to provide a reasonable basis for concluding that the drug:

(A) May be effective for its intended use in its intended patient population; or

(B) Would not expose the patients to whom the drug is to be administered to an unreasonable and significant additional risk of illness or injury.

(ii) For the purpose of this section, an "immediately life-threatening" disease means a stage of a disease in which there is a reasonable likelihood that death will occur within a matter of months or in which premature death is likely without early treatment.

(c) *Safeguards.* Treatment use of an investigational drug is [illegible]

e sponsor and investigators com-ring with the safeguards of the IND ocess, including the regulations gov-ning informed consent (21 CFR part and institutional review boards (21 R part 56) and the applicable provi-ns of part 312, including distribution the drug through qualified experts, intenance of adequate manufac-ring facilities, and submission of IND ety reports.

d) *Clinical hold.* FDA may place on nical hold a proposed or ongoing atment protocol or treatment IND accordance with §312.42.

FR 19476, May 22, 1987, as amended at 57 13248, Apr. 15, 1992]

2.35 Submissions for treatment use.

a) *Treatment protocol submitted by) sponsor.* Any sponsor of a clinical estigation of a drug who intends to nsor a treatment use for the drug ll submit to FDA a treatment pro-ol under §312.34 if the sponsor be-es the criteria of §312.34 are satis-l. If a protocol is not submitted ler §312.34, but FDA believes that protocol should have been sub-ted under this section, FDA may m the protocol to be submitted er §312.34. A treatment use under a atment protocol may begin 30 days r FDA receives the protocol or on lier notification by FDA that the atment use described in the protocol y begin.

) A treatment protocol is required ontain the following:

) The intended use of the drug.

i) An explanation of the rationale use of the drug, including, as appro-te, either a list of what available mens ordinarily should be tried be-using the investigational drug or explanation of why the use of the stigational drug is preferable to use of available marketed treat-ts.

f) A brief description of the criteria patient selection.

) The method of administration of drug and the dosages.

) A description of clinical proce-s, laboratory tests, or other meas- to monitor the effects of the drug to minimize risk.

(2) A treatment protocol is to be supported by the following:

(i) Informational brochure for supplying to each treating physician.

(ii) The technical information that is relevant to safety and effectiveness of the drug for the intended treatment purpose. Information contained in the sponsor's IND may be incorporated by reference.

(iii) A commitment by the sponsor to assure compliance of all participating investigators with the informed consent requirements of 21 CFR part 50.

(3) A licensed practioner who receives an investigational drug for treatment use under a treatment protocol is an "investigator" under the protocol and is responsible for meeting all applicable investigator responsibilities under this part and 21 CFR parts 50 and 56.

(b) *Treatment IND submitted by licensed practitioner.* (1) If a licensed medical practitioner wants to obtain an investigational drug subject to a controlled clinical trial for a treatment use, the practitioner should first attempt to obtain the drug from the sponsor of the controlled trial under a treatment protocol. If the sponsor of the controlled clinical investigation of the drug will not establish a treatment protocol for the drug under paragraph (a) of this section, the licensed medical practitioner may seek to obtain the drug from the sponsor and submit a treatment IND to FDA requesting authorization to use the investigational drug for treatment use. A treatment use under a treatment IND may begin 30 days after FDA receives the IND or on earlier notification by FDA that the treatment use under the IND may begin. A treatment IND is required to contain the following:

(i) A cover sheet (Form FDA 1571) meeting §312.23(a)(1).

(ii) Information (when not provided by the sponsor) on the drug's chemistry, manufacturing, and controls, and prior clinical and nonclinical experience with the drug submitted in accordance with §312.23. A sponsor of a clinical investigation subject to an IND who supplies an investigational drug to a licensed medical practitioner for purposes of a separate treatment clinical investigation shall be deemed to authorize the incorporation-by-reference of the technical information contained in the sponsor's IND into the medical practitioner's treatment IND.

(iii) A statement of the steps taken by the practitioner to obtain the drug under a treatment protocol from the drug sponsor.

(iv) A treatment protocol containing the same information listed in paragraph (a)(1) of this section.

(v) A statement of the practitioner's qualifications to use the investigational drug for the intended treatment use.

(vi) The practitioner's statement of familiarity with information on the drug's safety and effectiveness derived from previous clinical and nonclinical experience with the drug.

(vii) Agreement to report to FDA safety information in accordance with §312.32.

(2) A licensed practitioner who submits a treatment IND under this section is the sponsor-investigator for such IND and is responsible for meeting all applicable sponsor and investigator responsibilities under this part and 21 CFR parts 50 and 56.

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[52 FR 19477, May 22, 1987, as amended at 57 FR 13249, Apr. 15, 1992]

§312.36 Emergency use of an investigational new drug.

Need for an investigational drug may arise in an emergency situation that does not allow time for submission of an IND in accordance with §312.23 or §312.34. In such a case, FDA may authorize shipment of the drug for a specified use in advance of submission of an IND. A request for such authorization may be transmitted to FDA by telephone or other rapid communication means. For investigational biological drugs, the request should be directed to the Division of Biological Investigational New Drugs (HFB-230), Center for Biologics Evaluation and Research, 8800 Rockville Pike, Bethesda, MD 20892, 301-443-4864. For all other investigational drugs, the request for authorization should be directed to the Document Management and Reporting Branch (HFD-53), Center for Drug Evaluation and Research, 5600 Fishers Lane, Rockville, MD 20857, 301-443-4320. After normal working hours, eastern standard time, the request should be directed to the FDA Division of Emergency and Epidemiological Operations, 202-857-8400. Except in extraordinary circumstances, such authorization will be conditioned on the sponsor making an appropriate IND submission as soon as practicable after receiving the authorization.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11579, Mar. 29, 1990]

§312.38 Withdrawal of an IND.

(a) At any time a sponsor may withdraw an effective IND without prejudice.

(b) If an IND is withdrawn, FDA shall be so notified, all clinical investigations conducted under the IND shall be ended, all current investigators notified, and all stocks of the drug returned to the sponsor or otherwise disposed of at the request of the sponsor in accordance with §312.59.

(c) If an IND is withdrawn because of a safety reason, the sponsor shall promptly so inform FDA, all participating investigators, and all reviewing Institutional Review Boards, together with the reasons for such withdrawal.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

Subpart C—Administrative Actions

§312.40 General requirements for use of an investigational new drug in a clinical investigation.

(a) An investigational new drug may be used in a clinical investigation if the following conditions are met:

(1) The sponsor of the investigation submits an IND for the drug to FDA; the IND is in effect under paragraph (b) of this section; and the sponsor complies with all applicable requirements in this part and parts 50 and 56 with respect to the conduct of the clinical in-

(2) Each participating investigator conducts his or her investigation in compliance with the requirements of this part and parts 50 and 56.

(b) An IND goes into effect:

(1) Thirty days after FDA receives the IND, unless FDA notifies the sponsor that the investigations described in the IND are subject to a clinical hold under §312.42; or

(2) On earlier notification by FDA that the clinical investigations in the IND may begin. FDA will notify the sponsor in writing of the date it receives the IND.

(c) A sponsor may ship an investigational new drug to investigators named in the IND:

(1) Thirty days after FDA receives the IND; or

(2) On earlier FDA authorization to ship the drug.

(d) An investigator may not administer an investigational new drug to human subjects until the IND goes into effect under paragraph (b) of this section.

§312.41 Comment and advice on an IND.

(a) FDA may at any time during the course of the investigation communicate with the sponsor orally or in writing about deficiencies in the IND or about FDA's need for more data or information.

(b) On the sponsor's request, FDA will provide advice on specific matters relating to an IND. Examples of such advice may include advice on the adequacy of technical data to support an investigational plan, on the design of a clinical trial, and on whether proposed investigations are likely to produce the data and information that is needed to meet requirements for a marketing application.

(c) Unless the communication is accompanied by a clinical hold order under §312.42, FDA communications with a sponsor under this section are solely advisory and do not require any modification in the planned or ongoing clinical investigations or response to the agency.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§312.42 Clinical holds and requests for modification.

(a) *General.* A clinical hold is an order issued by FDA to the sponsor to delay a proposed clinical investigation or to suspend an ongoing investigation. The clinical hold order may apply to one or more of the investigations covered by an IND. When a proposed study is placed on clinical hold, subjects may not be given the investigational drug. When an ongoing study is placed on clinical hold, no new subjects may be recruited to the study and placed on the investigational drug; patients already in the study should be taken off therapy involving the investigational drug unless specifically permitted by FDA in the interest of patient safety.

(b) *Grounds for imposition of clinical hold*—(1) *Clinical hold of a Phase 1 study under an IND.* FDA may place a proposed or ongoing Phase 1 investigation on clinical hold if it finds that:

(i) Human subjects are or would be exposed to an unreasonable and significant risk of illness or injury;

(ii) The clinical investigators named in the IND are not qualified by reason of their scientific training and experience to conduct the investigation described in the IND;

(iii) The investigator brochure is misleading, erroneous, or materially incomplete; or

(iv) The IND does not contain sufficient information required under §312.23 to assess the risks to subjects of the proposed studies.

(2) *Clinical hold of a Phase 2 or 3 study under an IND.* FDA may place a proposed or ongoing Phase 2 or 3 investigation on clinical hold if it finds that:

(i) Any of the conditions in paragraph (b)(1)(i) through (iv) of this section apply; or

(ii) The plan or protocol for the investigation is clearly deficient in design to meet its stated objectives.

(3) Clinical hold of a treatment IND or treatment protocol.

(i) *Proposed use.* FDA may place a proposed treatment IND or treatment protocol on clinical hold if it is determined that:

(A) The pertinent criteria in §312.34(b) for permitting the treatment use to begin are not satisfied; or

(B) The treatment protocol or treatment IND does not contain the information required under §312.35 (a) or (b) to make the specified determination under §312.34(b).

(ii) *Ongoing use.* FDA may place an ongoing treatment protocol or treatment IND on clinical hold if it is determined that:

(A) There becomes available a comparable or satisfactory alternative drug or other therapy to treat that stage of the disease in the intended patient population for which the investigational drug is being used;

(B) The investigational drug is not under investigation in a controlled clinical trial under an IND in effect for the trial and not all controlled clinical trials necessary to support a marketing application have been completed, or a clinical study under the IND has been placed on clinical hold;

(C) The sponsor of the controlled clinical trial is not pursuing marketing approval with due diligence;

(D) If the treatment IND or treatment protocol is intended for a serious disease, there is insufficient evidence of safety and effectiveness to support such use; or

(E) If the treatment protocol or treatment IND was based on an immediately life-threatening disease, the available scientific evidence, taken as a whole, fails to provide a reasonable basis for concluding that the drug:

(*1*) May be effective for its intended use in its intended population; or

(*2*) Would not expose the patients to whom the drug is to be administered to an unreasonable and significant additional risk of illness or injury.

(iii) FDA may place a proposed or ongoing treatment IND or treatment protocol on clinical hold if it finds that any of the conditions in paragraph (b)(4)(i) through (b)(4)(viii) of this section apply.

(4) *Clinical hold of any study that is not designed to be adequate and well-controlled.* FDA may place a proposed or ongoing investigation that is not designed to be adequate and well-controlled on clinical hold if it finds that:

(i) Any of the conditions in paragraph (b)(1) or (b)(2) of this section apply; or

(ii) There is reasonable evidence the investigation that is not designed to be adequate and well-controlled is impeding enrollment in, or otherwise interfering with the conduct or completion of, a study that is designed to be an adequate and well-controlled investigation of the same or another investigational drug; or

(iii) Insufficient quantities of the investigational drug exist to adequately conduct both the investigation that is not designed to be adequate and well-controlled and the investigations that are designed to be adequate and well-controlled; or

(iv) The drug has been studied in one or more adequate and well-controlled investigations that strongly suggest lack of effectiveness; or

(v) Another drug under investigation or approved for the same indication and available to the same patient population has demonstrated a better potential benefit/risk balance; or

(vi) The drug has received marketing approval for the same indication in the same patient population; or

(vii) The sponsor of the study that is designed to be an adequate and well-controlled investigation is not actively pursuing marketing approval of the investigational drug with due diligence; or

(viii) The Commissioner determines that it would not be in the public interest for the study to be conducted or continued. FDA ordinarily intends that clinical holds under paragraphs (b)(4)(ii), (b)(4)(iii) and (b)(4)(v) of this section would only apply to additional enrollment in nonconcurrently controlled trials rather than eliminating continued access to individuals already receiving the investigational drug.

(5) *Clinical hold of any investigation involving an exception from informed consent under §50.24 of this chapter.* FDA

ıay place a proposed or ongoing investigation involving an exception from ıformed consent under §50.24 of this hapter on clinical hold if it is deterined that:

(i) Any of the conditions in paraaphs (b)(1) or (b)(2) of this section pply; or

(ii) The pertinent criteria in §50.24 of ıis chapter for such an investigation begin or continue are not submitted not satisfied.

(c) *Discussion of deficiency.* Whenever DA concludes that a deficiency exists a clinical investigation that may be ounds for the imposition of clinical ıld FDA will, unless patients are exısed to immediate and serious risk, tempt to discuss and satisfactorily solve the matter with the sponsor bere issuing the clinical hold order.

(d) *Imposition of clinical hold.* The inical hold order may be made by lephone or other means of rapid comunication or in writing. The clinical ıld order will identify the studies ıder the IND to which the hold aples, and will briefly explain the basis r the action. The clinical hold order ll be made by or on behalf of the Dision Director with responsibility for view of the IND. As soon as possible, ıd no more tha 30 days after imposiın of the clinical hold, the Division rector will provide the sponsor a ·itten explanation of the basis for the ıld.

(e) *Resumption of clinical investigaıns.* An investigation may only reme after FDA (usually the Division rector, or the Director's designee, th responsibility for review of the D) has notified the sponsor that the vestigation may proceed. Resumpın of the affected investigation(s) ll be authorized when the sponsor rrects the deficiency(ies) previously ed or otherwise satisfies the agency at the investigation(s) can proceed.)A may notify a sponsor of its deternation regarding the clinical hold by ephone or other means of rapid comınication. If a sponsor of an IND that s been placed on clinical hold reests in writing that the clinical hold removed and submits a complete reonse to the issue(s) identified in the nical hold order, FDA shall respond writing to the sponsor within 30-calendar days of receipt of the request a the complete response. FDA's response will either remove or maintain the clinical hold, and will state the reasons for such determination. Notwithstanding the 30-calendar day response time, a sponsor may not proceed with a clinical trial on which a clinical hold has been imposed until the sponsor has been notified by FDA that the hold has been lifted.

(f) *Appeal.* If the sponsor disagrees with the reasons cited for the clinical hold, the sponsor may request reconsideration of the decision in accordance with §312.48.

(g) *Conversion of IND on clinical hold to inactive status.* If all investigations covered by an IND remain on clinical hold for 1 year or more, the IND may be placed on inactive status by FDA under §312.45.

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 19477, May 22, 1987; 57 FR 13249, Apr. 15, 1992; 61 FR 51530, Oct. 2, 1996; 63 FR 68678, Dec. 14, 1998]

EFFECTIVE DATE NOTE: At 63 FR 68678, Dec. 14, 1998, §312.42 was amended by revising paragraph (e), effective Apr. 28, 1999. For the convenience of the user, the superseded text follows:

§312.42 Clinical holds and requests for modification.

* * * * *

(e) *Resumption of clinical investigations.* If, by the terms of the clinical hold order, resumption of the affected investigation is permitted without prior notification by FDA once a stated correction or modification is made, the investigation may proceed as soon as the correction or modification is made. In all other cases, an investigation may only resume after the Division Director (or the Director's designee) with responsibility for review of the IND has notified the sponsor that the investigation may proceed. In these cases resumption of the affected investigation(s) will be authorized when the sponsor corrects the deficiency(ies) previously cited or otherwise satisfied the agency that the investigation(s) can proceed. Resumption of a study may be authorized by telephone or other means of rapid communication.

* * * * *

§312.44 Termination.

(a) *General.* This section describes the procedures under which FDA may terminate an IND. If an IND is terminated, the sponsor shall end all clinical investigations conducted under the IND and recall or otherwise provide for the disposition of all unused supplies of the drug. A termination action may be based on deficiencies in the IND or in the conduct of an investigation under an IND. Except as provided in paragraph (d) of this section, a termination shall be preceded by a proposal to terminate by FDA and an opportunity for the sponsor to respond. FDA will, in general, only initiate an action under this section after first attempting to resolve differences informally or, when appropriate, through the clinical hold procedures described in §312.42.

(b) *Grounds for termination—(1) Phase 1.* FDA may propose to terminate an IND during Phase 1 if it finds that:

(i) Human subjects would be exposed to an unreasonable and significant risk of illness or injury.

(ii) The IND does not contain sufficient information required under §312.23 to assess the safety to subjects of the clinical investigations.

(iii) The methods, facilities, and controls used for the manufacturing, processing, and packing of the investigational drug are inadequate to establish and maintain appropriate standards of identity, strength, quality, and purity as needed for subject safety.

(iv) The clinical investigations are being conducted in a manner substantially different than that described in the protocols submitted in the IND.

(v) The drug is being promoted or distributed for commercial purposes not justified by the requirements of the investigation or permitted by §312.7.

(vi) The IND, or any amendment or report to the IND, contains an untrue statement of a material fact or omits material information required by this part.

(vii) The sponsor fails promptly to investigate and inform the Food and Drug Administration and all investigators of serious and unexpected adverse experiences in accordance with §312.32 or fails to make any other report required under this part.

(viii) The sponsor fails to submit an accurate annual report of the investigations in accordance with §312.33.

(ix) The sponsor fails to comply with any other applicable requirement of this part, part 50, or part 56.

(x) The IND has remained on inactive status for 5 years or more.

(xi) The sponsor fails to delay a proposed investigation under the IND or to suspend an ongoing investigation that has been placed on clinical hold under §312.42(b)(4).

(2) *Phase 2 or 3.* FDA may propose to terminate an IND during Phase 2 or Phase 3 if FDA finds that:

(i) Any of the conditions in paragraphs (b)(1)(i) through (b)(1)(xi) of this section apply; or

(ii) The investigational plan or protocol(s) is not reasonable as a bona fide scientific plan to determine whether or not the drug is safe and effective for use; or

(iii) There is convincing evidence that the drug is not effective for the purpose for which it is being investigated.

(3) FDA may propose to terminate a treatment IND if it finds that:

(i) Any of the conditions in paragraphs (b)(1)(i) through (x) of this section apply; or

(ii) Any of the conditions in §312.42(b)(3) apply.

(c) *Opportunity for sponsor response.* (1) If FDA proposes to terminate an IND, FDA will notify the sponsor in writing, and invite correction or explanation within a period of 30 days.

(2) On such notification, the sponsor may provide a written explanation or correction or may request a conference with FDA to provide the requested explanation or correction. If the sponsor does not respond to the notification within the allocated time, the IND shall be terminated.

(3) If the sponsor responds but FDA does not accept the explanation or correction submitted, FDA shall inform the sponsor in writing of the reason for the nonacceptance and provide the sponsor with an opportunity for a regulatory hearing before FDA under part 16 on the question of whether the IND should be terminated. The sponsor's request for a regulatory hearing must be made within 10 days of the sponsor's

receipt of FDA's notification of nonacceptance.

(d) *Immediate termination of IND.* Notwithstanding paragraphs (a) through (c) of this section, if at any time FDA concludes that continuation of the investigation presents an immediate and substantial danger to the health of individuals, the agency shall immediately, by written notice to the sponsor from the Director of the Center for Drug Evaluation and Research or the Director of the Center for Biologics Evaluation and Research, terminate the IND. An IND so terminated is subject to reinstatement by the Director on the basis of additional submissions that eliminate such danger. If an IND is terminated under this paragraph, the agency will afford the sponsor an opportunity for a regulatory hearing under part 16 on the question of whether the IND should be reinstated.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11579, Mar. 29, 1990; 57 FR 13249, Apr. 15, 1992]

§ 312.45 Inactive status.

(a) If no subjects are entered into clinical studies for a period of 2 years or more under an IND, or if all investigations under an IND remain on clinical hold for 1 year or more, the IND may be placed by FDA on inactive status. This action may be taken by FDA either on request of the sponsor or on FDA's own initiative. If FDA seeks to act on its own initiative under this section, it shall first notify the sponsor in writing of the proposed inactive status. Upon receipt of such notification, the sponsor shall have 30 days to respond as to why the IND should continue to remain active.

(b) If an IND is placed on inactive status, all investigators shall be so notified and all stocks of the drug shall be returned or otherwise disposed of in accordance with § 312.59.

(c) A sponsor is not required to submit annual reports to an IND on inactive status. An inactive IND is, however, still in effect for purposes of the public disclosure of data and information under § 312.130.

(d) A sponsor who intends to resume clinical investigation under an IND placed on inactive status shall submit a protocol amendment under § 312.30 containing the proposed general investigational plan for the coming year and appropriate protocols. If the protocol amendment relies on information previously submitted, the plan shall reference such information. Additional information supporting the proposed investigation, if any, shall be submitted in an information amendment. Notwithstanding the provisions of § 312.30, clinical investigations under an IND on inactive status may only resume (1) 30 days after FDA receives the protocol amendment, unless FDA notifies the sponsor that the investigations described in the amendment are subject to a clinical hold under § 312.42, or (2) on earlier notification by FDA that the clinical investigations described in the protocol amendment may begin.

(e) An IND that remains on inactive status for 5 years or more may be terminated under § 312.44.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.47 Meetings.

(a) *General.* Meetings between a sponsor and the agency are frequently useful in resolving questions and issues raised during the course of a clinical investigation. FDA encourages such meetings to the extent that they aid in the evaluation of the drug and in the solution of scientific problems concerning the drug, to the extent that FDA's resources permit. The general principle underlying the conduct of such meetings is that there should be free, full, and open communication about any scientific or medical question that may arise during the clinical investigation. These meetings shall be conducted and documented in accordance with part 10.

(b) *"End-of-Phase 2" meetings and meetings held before submission of a marketing application.* At specific times during the drug investigation process, meetings between FDA and a sponsor can be especially helpful in minimizing wasteful expenditures of time and money and thus in speeding the drug development and evaluation process. In particular, FDA has found meetings at the end of Phase 2 of an investigation (end-of-Phase 2 meetings) are of considerable assistance in planning later studies and that meetings held near completion of Phase 3 and before submission of a marketing application ("pre-NDA" meetings) are helpful in developing methods of presentation and submission of data in the marketing application that facilitate review and allow timely FDA response.

(1) *End-of-Phase 2 meetings*—(i) *Purpose.* The purpose of an end-of-phase 2 meeting is to determine the safety of proceeding to Phase 3, to evaluate the Phase 3 plan and protocols and the adequacy of current studies and plans to assess pediatric safety and effectiveness, and to identify any additional information necessary to support a marketing application for the uses under investigation.

(ii) *Eligibility for meeting.* While the end-of-Phase 2 meeting is designed primarily for IND's involving new molecular entities or major new uses of marketed drugs, a sponsor of any IND may request and obtain an end-of-Phase 2 meeting.

(iii) *Timing.* To be most useful to the sponsor, end-of-Phase 2 meetings should be held before major commitments of effort and resources to specific Phase 3 tests are made. The scheduling of an end-of-Phase 2 meeting is not, however, intended to delay the transition of an investigation from Phase 2 to Phase 3.

(iv) *Advance information.* At least 1 month in advance of an end-of-Phase 2 meeting, the sponsor should submit background information on the sponsor's plan for Phase 3, including summaries of the Phase 1 and 2 investigations, the specific protocols for Phase 3 clinical studies, plans for any additional nonclinical studies, plans for pediatric studies, including a time line for protocol finalization, enrollment, completion, and data analysis, or information to support any planned request for waiver or deferral of pediatric studies, and, if available, tentative labeling for the drug. The recommended contents of such a submission are described more fully in FDA Staff Manual Guide 4850.7 that is publicly available under FDA's public information regulations in part 20.

(v) *Conduct of meeting.* Arrangements for an end-of-Phase 2 meeting are to be made with the division in FDA's Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research which is responsible for review of the IND. The meeting will be scheduled by FDA at a time convenient to both FDA and the sponsor. Both the sponsor and FDA may bring consultants to the meeting. The meeting should be directed primarily at establishing agreement between FDA and the sponsor of the overall plan for Phase 3 and the objectives and design of particular studies. The adequacy of the technical information to support Phase 3 studies and/or a marketing application may also be discussed. FDA will also provide its best judgment, at that time, of the pediatric studies that will be required for the drug product and whether their submission will be deferred until after approval. Agreements reached at the meeting on these matters will be recorded in minutes of the conference that will be taken by FDA in accordance with § 10.65 and provided to the sponsor. The minutes along with any other written material provided to the sponsor will serve as a permanent record of any agreements reached. Barring a significant scientific development that requires otherwise, studies conducted in accordance with the agreement shall be presumed to be sufficient in objective and design for the purpose of obtaining marketing approval for the drug.

(2) *"Pre-NDA" and "pre-BLA" meetings.* FDA has found that delays associated with the initial review of a marketing application may be reduced by exchanges of information about a proposed marketing application. The primary purpose of this kind of exchange is to uncover any major unresolved problems, to identify those studies that the sponsor is relying on as adequate and well-controlled to establish the drug's effectiveness, to identify the status of ongoing or needed studies adequate to assess pediatric safety and effectiveness, to acquaint FDA reviewers with the general information to be

submitted in the marketing application (including technical information), to discuss appropriate methods for statistical analysis of the data, and to discuss the best approach to the presentation and formatting of data in the marketing application. Arrangements for such a meeting are to be initiated by the sponsor with the division responsible for review of the IND. To permit FDA to provide the sponsor with the most useful advice on preparing a marketing application, the sponsor should submit to FDA's reviewing division at least 1 month in advance of the meeting the following information:

(i) A brief summary of the clinical studies to be submitted in the application.

(ii) A proposed format for organizing the submission, including methods for presenting the data.

(iii) Information on the status of needed or ongoing pediatric studies.

(iv) Any other information for discussion at the meeting.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11580, Mar. 29, 1990; 63 FR 66669, Dec. 2, 1998]

§ 312.48 Dispute resolution.

(a) *General.* The Food and Drug Administration is committed to resolving differences between sponsors and FDA reviewing divisions with respect to requirements for IND's as quickly and amicably as possible through the cooperative exchange of information and views.

(b) *Administrative and procedural issues.* When administrative or procedural disputes arise, the sponsor should first attempt to resolve the matter with the division in FDA's Center for Drug Evaluation and Research or Center for Biologics Evaluation and Research which is responsible for review of the IND, beginning with the consumer safety officer assigned to the application. If the dispute is not resolved, the sponsor may raise the matter with the person designated as ombudsman, whose function shall be to investigate what has happened and to facilitate a timely and equitable resolution. Appropriate issues to raise with the ombudsman include resolving difficulties in scheduling meetings and obtaining timely replies to inquiries. Further details on this procedure are contained in FDA Staff Manual Guide 4820.7 that is publicly available under FDA's public information regulations in part 20.

(c) *Scientific and medical disputes.* (1) When scientific or medical disputes arise during the drug investigation process, sponsors should discuss the matter directly with the responsible reviewing officials. If necessary, sponsors may request a meeting with the appropriate reviewing officials and management representatives in order to seek a resolution. Requests for such meetings shall be directed to the director of the division in FDA's Center for Drug Evaluation and Research or Center for Biologics Evaluation and Research which is responsible for review of the IND. FDA will make every attempt to grant requests for meetings that involve important issues and that can be scheduled at mutually convenient times.

(2) The "end-of-Phase 2" and "pre-NDA" meetings described in §312.47(b) will also provide a timely forum for discussing and resolving scientific and medical issues on which the sponsor disagrees with the agency.

(3) In requesting a meeting designed to resolve a scientific or medical dispute, applicants may suggest that FDA seek the advice of outside experts, in which case FDA may, in its discretion, invite to the meeting one or more of its advisory committee members or other consultants, as designated by the agency. Applicants may rely on, and may bring to any meeting, their own consultants. For major scientific and medical policy issues not resolved by informal meetings, FDA may refer the matter to one of its standing advisory committees for its consideration and recommendations.

[52 FR 8831, Mar. 19, 1987, as amended at 55 FR 11580, Mar. 29, 1990]

Subpart D—Responsibilities of Sponsors and Investigators

§ 312.50 General responsibilities of sponsors.

Sponsors are responsible for selecting qualified investigators, providing them with the information they need to conduct an investigation properly, ensuring proper monitoring of the investigation(s), ensuring that the investigation(s) is conducted in accordance with the general investigational plan and protocols contained in the IND, maintaining an effective IND with respect to the investigations, and ensuring that FDA and all participating investigators are promptly informed of significant new adverse effects or risks with respect to the drug. Additional specific responsibilities of sponsors are described elsewhere in this part.

§ 312.52 Transfer of obligations to a contract research organization.

(a) A sponsor may transfer responsibility for any or all of the obligations set forth in this part to a contract research organization. Any such transfer shall be described in writing. If not all obligations are transferred, the writing is required to describe each of the obligations being assumed by the contract research organization. If all obligations are transferred, a general statement that all obligations have been transferred is acceptable. Any obligation not covered by the written description shall be deemed not to have been transferred.

(b) A contract research organization that assumes any obligation of a sponsor shall comply with the specific regulations in this chapter applicable to this obligation and shall be subject to the same regulatory action as a sponsor for failure to comply with any obligation assumed under these regulations. Thus, all references to "sponsor" in this part apply to a contract research organization to the extent that it assumes one or more obligations of the sponsor.

§ 312.53 Selecting investigators and monitors.

(a) *Selecting investigators.* A sponsor shall select only investigators qualified by training and experience as appropriate experts to investigate the drug.

(b) *Control of drug.* A sponsor shall ship investigational new drugs only to investigators participating in the investigation.

(c) *Obtaining information from the investigator.* Before permitting an investigator to begin participation in an investigation, the sponsor shall obtain the following:

(1) A signed investigator statement (Form FDA-1572) containing:

(i) The name and address of the investigator;

(ii) The name and code number, if any, of the protocol(s) in the IND identifying the study(ies) to be conducted by the investigator;

(iii) The name and address of any medical school, hospital, or other research facility where the clinical investigation(s) will be conducted;

(iv) The name and address of any clinical laboratory facilities to be used in the study;

(v) The name and address of the IRB that is responsible for review and approval of the study(ies);

(vi) A commitment by the investigator that he or she:

(*a*) Will conduct the study(ies) in accordance with the relevant, current protocol(s) and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, the rights, or welfare of subjects;

(*b*) Will comply with all requirements regarding the obligations of clinical investigators and all other pertinent requirements in this part;

(*c*) Will personally conduct or supervise the described investigation(s);

(*d*) Will inform any potential subjects that the drugs are being used for investigational purposes and will ensure that the requirements relating to obtaining informed consent (21 CFR part 50) and institutional review board review and approval (21 CFR part 56) are met;

(*e*) Will report to the sponsor adverse experiences that occur in the course of the investigation(s) in accordance with §312.64;

(*f*) Has read and understands the information in the investigator's brochure, including the potential risks and side effects of the drug; and

(*g*) Will ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the above commitments.

(vii) A commitment by the investigator that, for an investigation subject

to an institutional review requirement under part 56, an IRB that complies with the requirements of that part will be responsible for the initial and continuing review and approval of the clinical investigation and that the investigator will promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others, and will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to the human subjects.

(viii) A list of the names of the subinvestigators (e.g., research fellows, residents) who will be assisting the investigator in the conduct of the investigation(s).

(2) *Curriculum vitae.* A curriculum vitae or other statement of qualifications of the investigator showing the education, training, and experience that qualifies the investigator as an expert in the clinical investigation of the drug for the use under investigation.

(3) *Clinical protocol.* (i) For Phase 1 investigations, a general outline of the planned investigation including the estimated duration of the study and the maximum number of subjects that will be involved.

(ii) For Phase 2 or 3 investigations, an outline of the study protocol including an approximation of the number of subjects to be treated with the drug and the number to be employed as controls, if any; the clinical uses to be investigated; characteristics of subjects by age, sex, and condition; the kind of clinical observations and laboratory tests to be conducted; the estimated duration of the study; and copies or a description of case report forms to be used.

(4) *Financial disclosure information.* Sufficient accurate financial information to allow the sponsor to submit complete and accurate certification or disclosure statements required under part 54 of this chapter. The sponsor shall obtain a commitment from the clinical investigator to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

(d) *Selecting monitors.* A sponsor shall select a monitor qualified by training and experience to monitor the progress of the investigation.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 61 FR 57280, Nov. 5, 1996; 63 FR 5252, Feb. 2, 1998]

§ 312.54 Emergency research under § 50.24 of this chapter.

(a) The sponsor shall monitor the progress of all investigations involving an exception from informed consent under § 50.24 of this chapter. When the sponsor receives from the IRB information concerning the public disclosures required by § 50.24(a)(7)(ii) and (a)(7)(iii) of this chapter, the sponsor promptly shall submit to the IND file and to Docket Number 95S-0158 in the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857, copies of the information that was disclosed, identified by the IND number.

(b) The sponsor also shall monitor such investigations to identify when an IRB determines that it cannot approve the research because it does not meet the criteria in the exception in § 50.24(a) of this chapter or because of other relevant ethical concerns. The sponsor promptly shall provide this information in writing to FDA, investigators who are asked to participate in this or a substantially equivalent clinical investigation, and other IRB's that are asked to review this or a substantially equivalent investigation.

[61 FR 51530, Oct. 2, 1996]

§ 312.55 Informing investigators.

(a) Before the investigation begins, a sponsor (other than a sponsor-investigator) shall give each participating clinical investigator an investigator brochure containing the information described in § 312.23(a)(5).

(b) The sponsor shall, as the overall investigation proceeds, keep each participating investigator informed of new observations discovered by or reported to the sponsor on the drug, particularly with respect to adverse effects and safe use. Such information may be distributed to investigators by means of periodically revised investigator brochures, reprints or published studies, reports or letters to clinical investigators, or other appropriate means. Important safety information is required to be relayed to investigators in accordance with § 312.32.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.56 Review of ongoing investigations.

(a) The sponsor shall monitor the progress of all clinical investigations being conducted under its IND.

(b) A sponsor who discovers that an investigator is not complying with the signed agreement (Form FDA-1572), the general investigational plan, or the requirements of this part or other applicable parts shall promptly either secure compliance or discontinue shipments of the investigational new drug to the investigator and end the investigator's participation in the investigation. If the investigator's participation in the investigation is ended, the sponsor shall require that the investigator dispose of or return the investigational drug in accordance with the requirements of § 312.59 and shall notify FDA.

(c) The sponsor shall review and evaluate the evidence relating to the safety and effectiveness of the drug as it is obtained from the investigator. The sponsors shall make such reports to FDA regarding information relevant to the safety of the drug as are required under § 312.32. The sponsor shall make annual reports on the progress of the investigation in accordance with § 312.33.

(d) A sponsor who determines that its investigational drug presents an unreasonable and significant risk to subjects shall discontinue those investigations that present the risk, notify FDA, all institutional review boards, and all investigators who have at any time participated in the investigation of the discontinuance, assure the disposition of all stocks of the drug outstanding as required by § 312.59, and furnish FDA with a full report of the sponsor's actions. The sponsor shall discontinue the investigation as soon as possible, and in no event later than 5 working days after making the determination that the investigation should be discontinued. Upon request, FDA will confer with a sponsor on the need to discontinue an investigation.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.57 Recordkeeping and record retention.

(a) A sponsor shall maintain adequate records showing the receipt, shipment, or other disposition of the investigational drug. These records are required to include, as appropriate, the name of the investigator to whom the drug is shipped, and the date, quantity, and batch or code mark of each such shipment.

(b) A sponsor shall maintain complete and accurate records showing any financial interest in § 54.4(a)(3)(i), (a)(3)(ii), (a)(3)(iii), and (a)(3)(iv) of this chapter paid to clinical investigators by the sponsor of the covered study. A sponsor shall also maintain complete and accurate records concerning all other financial interests of investigators subject to part 54 of this chapter.

(c) A sponsor shall retain the records and reports required by this part for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has been so notified.

(d) A sponsor shall retain reserve samples of any test article and reference standard identified in, and used in any of the bioequivalence or bioavailability studies described in, § 320.38 or § 320.63 of this chapter, and release the reserve samples to FDA upon request, in accordance with, and for the period specified in § 320.38.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 58 FR 25926, Apr. 28, 1993; 63 FR 5252, Feb. 2, 1998]

§ 312.58 Inspection of sponsor's records and reports.

(a) *FDA inspection.* A sponsor shall upon request from any properly authorized officer or employee of the Food and Drug Administration, at reasonable times, permit such officer or employee to have access to and copy and verify any records and reports relating to a clinical investigation conducted under this part. Upon written request by FDA, the sponsor shall submit the records or reports (or copies of them) to FDA. The sponsor shall discontinue shipments of the drug to any investigator who has failed to maintain or make available records or reports of the investigation as required by this part.

(b) *Controlled substances.* If an investigational new drug is a substance listed in any schedule of the Controlled Substances Act (21 U.S.C. 801; 21 CFR part 1308), records concerning shipment, delivery, receipt, and disposition of the drug, which are required to be kept under this part or other applicable parts of this chapter shall, upon the request of a properly authorized employee of the Drug Enforcement Administration of the U.S. Department of Justice, be made available by the investigator or sponsor to whom the request is made, for inspection and copying. In addition, the sponsor shall assure that adequate precautions are taken, including storage of the investigational drug in a securely locked, substantially constructed cabinet, or other securely locked, substantially constructed enclosure, access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution.

§ 312.59 Disposition of unused supply of investigational drug.

The sponsor shall assure the return of all unused supplies of the investigational drug from each individual investigator whose participation in the investigation is discontinued or terminated. The sponsor may authorize alternative disposition of unused supplies of the investigational drug provided this alternative disposition does not expose humans to risks from the drug. The sponsor shall maintain written records of any disposition of the drug in accordance with § 312.57.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.60 General responsibilities of investigators.

An investigator is responsible for ensuring that an investigation is conducted according to the signed investigator statement, the investigational plan, and applicable regulations; for protecting the rights, safety, and welfare of subjects under the investigator's care; and for the control of drugs under investigation. An investigator shall, in accordance with the provisions of part 50 of this chapter, obtain the informed consent of each human subject to whom the drug is administered, except as provided in §§ 50.23 or 50.24 of this chapter. Additional specific responsibilities of clinical investigators are set forth in this part and in parts 50 and 56 of this chapter.

[52 FR 8831, Mar. 19, 1987, as amended at 61 FR 51530, Oct. 2, 1996]

§ 312.61 Control of the investigational drug.

An investigator shall administer the drug only to subjects under the investigator's personal supervision or under the supervision of a subinvestigator responsible to the investigator. The investigator shall not supply the investigational drug to any person not authorized under this part to receive it.

§ 312.62 Investigator recordkeeping and record retention.

(a) *Disposition of drug.* An investigator is required to maintain adequate records of the disposition of the drug, including dates, quantity, and use by subjects. If the investigation is terminated, suspended, discontinued, or completed, the investigator shall return the unused supplies of the drug to the sponsor, or otherwise provide for disposition of the unused supplies of the drug under § 312.59.

(b) *Case histories.* An investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

(c) *Record retention.* An investigator shall retain records required to be maintained under this part for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 61 FR 57280, Nov. 5, 1996]

§ 312.64 Investigator reports.

(a) *Progress reports.* The investigator shall furnish all reports to the sponsor of the drug who is responsible for collecting and evaluating the results obtained. The sponsor is required under § 312.33 to submit annual reports to FDA on the progress of the clinical investigations.

(b) *Safety reports.* An investigator shall promptly report to the sponsor any adverse effect that may reasonably be regarded as caused by, or probably caused by, the drug. If the adverse effect is alarming, the investigator shall report the adverse effect immediately.

(c) *Final report.* An investigator shall provide the sponsor with an adequate report shortly after completion of the investigator's participation in the investigation.

(d) *Financial disclosure reports.* The clinical investigator shall provide the sponsor with sufficient accurate financial information to allow an applicant to submit complete and accurate certification or disclosure statements as required under part 54 of this chapter. The clinical investigator shall promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 63 FR 5252, Feb. 2, 1998]

§ 312.66 Assurance of IRB review.

An investigator shall assure that an IRB that complies with the requirements set forth in part 56 will be responsible for the initial and continuing review and approval of the proposed clinical study. The investigator shall also assure that he or she will promptly report to the IRB all changes in the research activity and all unanticipated problems involving risk to human subjects or others, and that he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910–0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987]

§ 312.68 Inspection of investigator's records and reports.

An investigator shall upon request from any properly authorized officer or employee of FDA, at reasonable times, permit such officer or employee to have access to, and copy and verify any records or reports made by the investigator pursuant to § 312.62. The investigator is not required to divulge subject names unless the records of particular individuals require a more detailed study of the cases, or unless there is reason to believe that the records do not represent actual case studies, or do not represent actual results obtained.

§ 312.69 Handling of controlled substances.

If the investigational drug is subject to the Controlled Substances Act, the

investigator shall take adequate precautions, including storage of the investigational drug in a securely locked, substantially constructed cabinet, or other securely locked, substantially constructed enclosure, access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution.

§ 312.70 Disqualification of a clinical investigator.

(a) If FDA has information indicating that an investigator (including a sponsor-investigator) has repeatedly or deliberately failed to comply with the requirements of this part, part 50, or part 56 of this chapter, or has submitted to FDA or to the sponsor false information in any required report, the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research will furnish the investigator written notice of the matter complained of and offer the investigator an opportunity to explain the matter in writing, or, at the option of the investigator, in an informal conference. If an explanation is offered but not accepted by the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research, the investigator will be given an opportunity for a regulatory hearing under part 16 on the question of whether the investigator is entitled to receive investigational new drugs.

(b) After evaluating all available information, including any explanation presented by the investigator, if the Commissioner determines that the investigator has repeatedly or deliberately failed to comply with the requirements of this part, part 50, or part 56 of this chapter, or has deliberately or repeatedly submitted false information to FDA or to the sponsor in any required report, the Commissioner will notify the investigator and the sponsor of any investigation in which the investigator has been named as a participant that the investigator is not entitled to receive investigational drugs. The notification will provide a statement of basis for such determination.

(c) Each IND and each approved application submitted under part 314 containing data reported by an investigator who has been determined to be ineligible to receive investigational drugs will be examined to determine whether the investigator has submitted unreliable data that are essential to the continuation of the investigation or essential to the approval of any marketing application.

(d) If the Commissioner determines, after the unreliable data submitted by the investigator are eliminated from consideration, that the data remaining are inadequate to support a conclusion that it is reasonably safe to continue the investigation, the Commissioner will notify the sponsor who shall have an opportunity for a regulatory hearing under part 16. If a danger to the public health exists, however, the Commissioner shall terminate the IND immediately and notify the sponsor of the determination. In such case, the sponsor shall have an opportunity for a regulatory hearing before FDA under part 16 on the question of whether the IND should be reinstated.

(e) If the Commissioner determines, after the unreliable data submitted by the investigator are eliminated from consideration, that the continued approval of the drug product for which the data were submitted cannot be justified, the Commissioner will proceed to withdraw approval of the drug product in accordance with the applicable provisions of the act.

(f) An investigator who has been determined to be ineligible to receive investigational drugs may be reinstated as eligible when the Commissioner determines that the investigator has presented adequate assurances that the investigator will employ investigational drugs solely in compliance with the provisions of this part and of parts 50 and 56.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8031, Mar. 19, 1987, as amended at 52 FR 23001, June 17, 1987; 55 FR 11580, Mar. 29, 1990; 62 FR 46876, Sept. 5, 1997]

Subpart E—Drugs Intended to Treat Life-threatening and Severely-debilitating Illnesses

AUTHORITY: 21 U.S.C. 351, 352, 353, 355, 371; 42 U.S.C. 262.

SOURCE: 53 FR 41523, Oct. 21, 1988, unless otherwise noted.

§ 312.80 Purpose.

The purpose of this section is to establish procedures designed to expedite the development, evaluation, and marketing of new therapies intended to treat persons with life-threatening and severely-debilitating illnesses, especially where no satisfactory alternative therapy exists. As stated in § 314.105(c) of this chapter, while the statutory standards of safety and effectiveness apply to all drugs, the many kinds of drugs that are subject to them, and the wide range of uses for those drugs, demand flexibility in applying the standards. The Food and Drug Administration (FDA) has determined that it is appropriate to exercise the broadest flexibility in applying the statutory standards, while preserving appropriate guarantees for safety and effectiveness. These procedures reflect the recognition that physicians and patients are generally willing to accept greater risks or side effects from products that treat life-threatening and severely-debilitating illnesses, than they would accept from products that treat less serious illnesses. These procedures also reflect the recognition that the benefits of the drug need to be evaluated in light of the severity of the disease being treated. The procedure outlined in this section should be interpreted consistent with that purpose.

§ 312.81 Scope.

This section applies to new drug and biological products that are being studied for their safety and effectiveness in treating life-threatening or severely-debilitating diseases.

(a) For purposes of this section, the term "life-threatening" means:

(1) Diseases or conditions where the likelihood of death is high unless the course of the disease is interrupted; and

(2) Diseases or conditions with potentially fatal outcomes, where the end point of clinical trial analysis is survival.

(b) For purposes of this section, the term "severely debilitating" means diseases or conditions that cause major irreversible morbidity.

(c) Sponsors are encouraged to consult with FDA on the applicability of these procedures to specific products.

[53 FR 41523, Oct. 21, 1988, as amended at 64 FR 401, Jan. 5, 1999]

EFFECTIVE DATE NOTE: At 64 FR 401, Jan. 5, 1999, § 312.80 was amended by removing ", antibiotic," from the introductory text, effective May 20, 1999.

§ 312.82 Early consultation.

For products intended to treat life-threatening or severely-debilitating illnesses, sponsors may request to meet with FDA-reviewing officials early in the drug development process to review and reach agreement on the design of necessary preclinical and clinical studies. Where appropriate, FDA will invite to such meetings one or more outside expert scientific consultants or advisory committee members. To the extent FDA resources permit, agency reviewing officials will honor requests for such meetings.

(a) *Pre-investigational new drug (IND) meetings.* Prior to the submission of the initial IND, the sponsor may request a meeting with FDA-reviewing officials. The primary purpose of this meeting is to review and reach agreement on the design of animal studies needed to initiate human testing. The meeting may also provide an opportunity for discussing the scope and design of phase 1 testing, plans for studying the drug product in pediatric populations, and the best approach for presentation and formatting of data in the IND.

(b) *End-of-phase 1 meetings.* When data from phase 1 clinical testing are available, the sponsor may again request a meeting with FDA-reviewing officials. The primary purpose of this meeting is to review and reach agreement on the design of phase 2 controlled clinical trials, with the goal that such testing will be adequate to provide sufficient data on the drug's safety and effectiveness to support a decision on its approvability for marketing, and to discuss the need for, as well as the design and timing of, studies of the drug in pediatric patients. For drugs for life-threatening diseases, FDA will provide its best judgment, at that time, whether pediatric studies will be required and whether their submission will be

leferred until after approval. The pro-
:edures outlined in §312.47(b)(1) with
'espect to end-of-phase 2 conferences,
ncluding documentation of agree-
nents reached, would also be used for
:nd-of-phase 1 meetings.

53 FR 41523, Oct. 21, 1988, as amended at 63
'R 66669, Dec. 2, 1998]

312.83 Treatment protocols.

If the preliminary analysis of phase 2
est results appears promising, FDA
nay ask the sponsor to submit a treat-
:ent protocol to be reviewed under the
rocedures and criteria listed in
§312.34 and 312.35. Such a treatment
rotocol if requested and granted,
'ould normally remain in effect while
he complete data necessary for a mar-
eting application are being assembled
y the sponsor and reviewed by FDA
nless grounds exist for clinical hold
f ongoing protocols, as provided in
312.42(b)(3)(ii)).

312.84 Risk-benefit analysis in review of marketing applications for drugs to treat life-threatening and severely-debilitating illnesses.

(a) FDA's application of the statu-
ory standards for marketing approval
ill recognize the need for a medical
sk-benefit judgment in making the
nal decision on approvability. As part
this evaluation, consistent with the
atement of purpose in §312.80, FDA
ill consider whether the benefits of
ie drug outweigh the known and po-
ntial risks of the drug and the need
answer remaining questions about
sks and benefits of the drug, taking
to consideration the severity of the
sease and the absence of satisfactory
lternative therapy.

(b) In making decisions on whether
grant marketing approval for prod-
ts that have been the subject of an
d-of-phase 1 meeting under §312.82,
)A will usually seek the advice of
tside expert scientific consultants or
visory committees. Upon the filing
such a marketing application under
14.101 or part 601 of this chapter, FDA
ll notify the members of the relevant
nding advisory committee of the ap-
cation's filing and its availability
review.

(c) If FDA concludes that the data
:sented are not sufficient for mar-
keting approval, FDA will issue (for a drug) a not approvable letter pursuant to §314.120 of this chapter, or (for a biologic) a deficiencies letter consistent with the biological product licensing procedures. Such letter, in describing the deficiencies in the application, will address why the results of the research design agreed to under §312.82, or in subsequent meetings, have not provided sufficient evidence for marketing approval. Such letter will also describe any recommendations made by the advisory committee regarding the application.

(d) Marketing applications submitted under the procedures contained in this section will be subject to the requirements and procedures contained in part 314 or part 600 of this chapter, as well as those in this subpart.

§312.85 Phase 4 studies.

Concurrent with marketing approval, FDA may seek agreement from the sponsor to conduct certain postmarketing (phase 4) studies to delineate additional information about the drug's risks, benefits, and optimal use. These studies could include, but would not be limited to, studying different doses or schedules of administration than were used in phase 2 studies, use of the drug in other patient populations or other stages of the disease, or use of the drug over a longer period of time.

§312.86 Focused FDA regulatory research.

At the discretion of the agency, FDA may undertake focused regulatory research on critical rate-limiting aspects of the preclinical, chemical/manufacturing, and clinical phases of drug development and evaluation. When initiated, FDA will undertake such research efforts as a means for meeting a public health need in facilitating the development of therapies to treat life-threatening or severely debilitating illnesses.

§312.87 Active monitoring of conduct and evaluation of clinical trials.

For drugs covered under this section, the Commissioner and other agency officials will monitor the progress of the conduct and evaluation of clinical trials and be involved in facilitating their appropriate progress.

§312.88 Safeguards for patient safety.

All of the safeguards incorporated within parts 50, 56, 312, 314, and 600 of this chapter designed to ensure the safety of clinical testing and the safety of products following marketing approval apply to drugs covered by this section. This includes the requirements for informed consent (part 50 of this chapter) and institutional review boards (part 56 of this chapter). These safeguards further include the review of animal studies prior to initial human testing (§312.23), and the monitoring of adverse drug experiences through the requirements of IND safety reports (§312.32), safety update reports during agency review of a marketing application (§314.50 of this chapter), and postmarketing adverse reaction reporting (§314.80 of this chapter).

Subpart F—Miscellaneous

§312.110 Import and export requirements.

(a) *Imports.* An investigational new drug offered for import into the United States complies with the requirements of this part if it is subject to an IND that is in effect for it under §312.40 and: (1) The consignee in the United States is the sponsor of the IND; (2) the consignee is a qualified investigator named in the IND; or (3) the consignee is the domestic agent of a foreign sponsor, is responsible for the control and distribution of the investigational drug, and the IND identifies the consignee and describes what, if any, actions the consignee will take with respect to the investigational drug.

(b) *Exports.* An investigational new drug intended for export from the United States complies with the requirements of this part as follows:

(1) If an IND is in effect for the drug under §312.40 and each person who receives the drug is an investigator named in the application; or

(2) If FDA authorizes shipment of the drug for use in a clinical investigation. Authorization may be obtained as follows:

(i) Through submission to the International Affairs Staff (HFY-50), Associate Commissioner for Health Affairs, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, of a written request from the person that seeks to export the drug. A request must provide adequate information about the drug to satisfy FDA that the drug is appropriate for the proposed investigational use in humans, that the drug will be used for investigational purposes only, and that the drug may be legally used by that consignee in the importing country for the proposed investigational use. The request shall specify the quantity of the drug to be shipped per shipment and the frequency of expected shipments. If FDA authorizes exportation under this paragraph, the agency shall concurrently notify the government of the importing country of such authorization.

(ii) Through submission to the International Affairs Staff (HFY-50), Associate Commissioner for Health Affairs, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, of a formal request from an authorized official of the government of the country to which the drug is proposed to be shipped. A request must specify that the foreign government has adequate information about the drug and the proposed investigational use, that the drug will be used for investigational purposes only, and that the foreign government is satisfied that the drug may legally be used by the intended consignee in that country. Such a request shall specify the quantity of drug to be shipped per shipment and the frequency of expected shipments.

(iii) Authorization to export an investigational drug under paragraph (b)(2)(i) or (ii) of this section may be revoked by FDA if the agency finds that the conditions underlying its authorization are not longer met.

(3) This paragraph applies only where the drug is to be used for the purpose of clinical investigation.

(4) This paragraph does not apply to the export of new drugs (including biological products, antibiotic drugs, and insulin) approved or authorized for export under section 802 of the act (21 U.S.C. 382) or section 351(h)(1)(A) of the

Public Health Service Act (42 U.S.C. 262(h)(J)(A)).

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 64 FR 401, Jan. 5, 1999]

EFFECTIVE DATE NOTE: At 64 FR 401, Jan. 5, 1999, §312.110 was amended by revising paragraph (b)(4) and by removing paragraph (b)(5), effective May 20, 1999. For the convenience of the user, the superseded text follows:

§312.110 Import and export requirements.

* * * * *

(b) * * *

(4) This paragraph does not apply to the export of an antibiotic drug product shipped in accordance with the provisions of section 801(d) of the act.

(5) This paragraph does not apply to the export of new drugs (including biological products) approved for export under section 802 of the act or section 351(h)(1)(A) of the Public Health Service Act.

* * * * *

§312.120 Foreign clinical studies not conducted under an IND.

(a) *Introduction.* This section describes the criteria for acceptance by FDA of foreign clinical studies not conducted under an IND. In general, FDA accepts such studies provided they are well designed, well conducted, performed by qualified investigators, and conducted in accordance with ethical principles acceptable to the world community. Studies meeting these criteria may be utilized to support clinical investigations in the United States and/or marketing approval. Marketing approval of a new drug based solely on foreign clinical data is governed by §314.106.

(b) *Data submissions.* A sponsor who wishes to rely on a foreign clinical study to support an IND or to support an application for marketing approval shall submit to FDA the following information:

(1) A description of the investigator's qualifications;

(2) A description of the research facilities;

(3) A detailed summary of the protocol and results of the study; and, should FDA request, case records maintained by the investigator or additional background data such as hospital or other institutional records;

(4) A description of the drug substance and drug product used in the study, including a description of components, formulation, specifications, and bioavailability of the specific drug product used in the clinical study, if available; and

(5) If the study is intended to support the effectiveness of a drug product, information showing that the study is adequate and well controlled under §314.126.

(c) *Conformance with ethical principles.* (1) Foreign clinical research is required to have been conducted in accordance with the ethical principles stated in the "Declaration of Helsinki" (see paragraph (c)(4) of this section) or the laws and regulations of the country in which the research was conducted, whichever represents the greater protection of the individual.

(2) For each foreign clinical study submitted under this section, the sponsor shall explain how the research conformed to the ethical principles contained in the "Declaration of Helsinki" or the foreign country's standards, whichever were used. If the foreign country's standards were used, the sponsor shall explain in detail how those standards differ from the "Declaration of Helsinki" and how they offer greater protection.

(3) When the research has been approved by an independent review committee, the sponsor shall submit to FDA documentation of such review and approval, including the names and qualifications of the members of the committee. In this regard, a "review committee" means a committee composed of scientists and, where practicable, individuals who are otherwise qualified (e.g., other health professionals or laymen). The investigator may not vote on any aspect of the review of his or her protocol by a review committee.

(4) The "Declaration of Helsinki" states as follows:

RECOMMENDATIONS GUIDING PHYSICIANS IN BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS

Introduction

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfillment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. Basic Principles

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.

6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a

dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.

Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. Medical Research Combined with Professional Care (Clinical Research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgment it offers hope of saving life, reestablishing health or alleviating suffering.

2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

3. In any medical study, every patient—including those of a control group, if any—should be assured of the best proven diagnostic and therapeutic method.

4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).

6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

I. Non-Therapeutic Biomedical Research Involving Human Subjects (Non-Clinical Biomedical Research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

2. The subjects should be volunteers—either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3. The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 56 FR 22113, May 14, 1991; 64 FR 401, Jan. 5, 1999]

EFFECTIVE DATE NOTE: At 64 FR 401, Jan. 5, 1999, §312.120 was amended by removing "or antibiotic drug" from the last sentence of paragraph (a), effective May 20, 1999.

§ 312.130 Availability for public disclosure of data and information in an IND.

(a) The existence of an investigational new drug application will not be disclosed by FDA unless it has previously been publicly disclosed or acknowledged.

(b) The availability for public disclosure of all data and information in an investigational new drug application for a new drug will be handled in accordance with the provisions established in §314.430 for the confidentiality of data and information in applications submitted in part 314. The availability for public disclosure of all data and information in an investigational new drug application for a biological product will be governed by the provisions of §§601.50 and 601.51.

(c) Notwithstanding the provisions of §314.430, FDA shall disclose upon request to an individual to whom an investigational new drug has been given a copy of any IND safety report relating to the use in the individual.

(d) The availability of information required to be publicly disclosed for investigations involving an exception from informed consent under §50.24 of this chapter will be handled as follows: Persons wishing to request the publicly disclosable information in the IND that was required to be filed in Docket Number 95S-0158 in the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857, shall submit a request under the Freedom of Information Act.

[52 FR 8831, Mar. 19, 1987, Redesignated at 53 FR 41523, Oct. 21, 1988, as amended at 61 FR 51530, Oct. 2, 1996; 64 FR 401, Jan. 5, 1999]

EFFECTIVE DATE NOTE: At 64 FR 401, Jan. 5, 1999, §312.130 was amended by removing "or antibiotic drug" from paragraph (b), effective May 20, 1999.

§ 312.140 Address for correspondence.

(a) Except as provided in paragraph (b) of this section, a sponsor shall send an initial IND submission to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, Park Bldg., Rm. 214, 12420 Parklawn Dr., Rockville, MD 20852. On receiving the IND, FDA will inform the sponsor which one of the divisions in the Center for Drug Evaluation and Research or the Center for Biologics Evaluation and Research is responsible for the IND. Amendments, reports, and other correspondence relating to matters covered by the IND should be directed to the appropriate division. The outside wrapper of each submission shall state what is contained in the submission, for example, "IND Application", "Protocol Amendment", etc.

(b) Applications for the products listed below should be submitted to the Division of Biological Investigational New Drugs (HFB-230), Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892. (1) Products subject to the licensing provisions of the Public Health Service Act of July 1, 1944 (58 Stat. 682, as amended (42 U.S.C. 201 *et seq.*)) or subject to part 600; (2) ingredients packaged together with containers intended for the collection, processing, or storage of blood or blood components; (3) urokinase products; (4) plasma volume expanders and hydroxyethyl starch for leukapheresis; and (5) coupled antibodies, i.e., products that consist of an antibody component coupled with a drug or radionuclide component in which both components provide a pharmacological effect but the biological component determines the [illegible]

(c) All correspondence relating to biological products for human use which are also radioactive drugs shall be submitted to the Division of Oncology and Radiopharmaceutical Drug Products (HFD-150), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, except that applications for coupled antibodies shall be submitted in accordance with paragraph (b) of this section.

(d) All correspondence relating to export of an investigational drug under §312.110(b)(2) shall be submitted to the International Affairs Staff (HFY-50), Office of Health Affairs, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857.

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[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987; 55 FR 11580, Mar. 29, 1990]

§ 312.145 Guidelines.

(a) FDA has made available guidelines under §10.90(b) to help persons to comply with certain requirements of this part.

(b) The Center for Drug Evaluation and Research and the Center for Biologics Evaluation and Research maintain lists of guidelines that apply to the Centers' regulations. The lists state how a person can obtain a copy of each guideline. A request for a copy of the lists should be directed to the CDER Executive Secretariat Staff (HFD-8), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, for drug products, and the Congressional, Consumer, and International Affairs Staff (HFB-142), Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892, for biological products.

[52 FR 8831, Mar. 19, 1987, as amended at 55 FR 11580, Mar. 29, 1990; 56 FR 3776, Jan. 31, 1991; 57 FR 10814, Mar. 31, 1992]

Subpart G—Drugs for Investigational Use in Laboratory Research Animals or In Vitro Tests

§ 312.160 Drugs for investigational use in laboratory research animals or in vitro tests.

(a) *Authorization to ship.* (1)(i) A person may ship a drug intended solely for tests in vitro or in animals used only for laboratory research purposes if it is labeled as follows:

CAUTION: Contains a new drug for investigational use only in laboratory research animals, or for tests in vitro. Not for use in humans.

(ii) A person may ship a biological product for investigational in vitro diagnostic use that is listed in §312.2(b)(2)(ii) if it is labeled as follows:

CAUTION: Contains a biological product for investigational in vitro diagnostic tests only.

(2) A person shipping a drug under paragraph (a) of this section shall use due diligence to assure that the consignee is regularly engaged in conducting such tests and that the shipment of the new drug will actually be used for tests in vitro or in animals used only for laboratory research.

(3) A person who ships a drug under paragraph (a) of this section shall maintain adequate records showing the name and post office address of the expert to whom the drug is shipped and the date, quantity, and batch or code mark of each shipment and delivery. Records of shipments under paragraph (a)(1)(i) of this section are to be maintained for a period of 2 years after the shipment. Records and reports of data and shipments under paragraph (a)(1)(ii) of this section are to be maintained in accordance with §312.57(b). The person who ships the drug shall upon request from any properly authorized officer or employee of the Food and Drug Administration, at reasonable times, permit such officer or employee to have access to and copy and verify records required to be maintained under this section.

(b) *Termination of authorization to ship.* FDA may terminate authorization to ship a drug under this section if it finds that:

(1) The sponsor of the investigation has failed to comply with any of the conditions for shipment established under this section; or

(2) The continuance of the investigation is unsafe or otherwise contrary to the public interest or the drug is used for purposes other than bona fide scientific investigation. FDA will notify the person shipping the drug of its finding and invite immediate correction. If correction is not immediately made, the person shall have an opportunity for a regulatory hearing before FDA pursuant to part 16.

(c) *Disposition of unused drug.* The person who ships the drug under paragraph (a) of this section shall assure the return of all unused supplies of the drug from individual investigators whenever the investigation discontinues or the investigation is terminated. The person who ships the drug may authorize in writing alternative disposition of unused supplies of the drug provided this alternative disposition does not expose humans to risks from the drug, either directly or indirectly (e.g., through food-producing animals). The shipper shall maintain records of any alternative disposition.

(Collection of information requirements approved by the Office of Management and Budget under control number 0910-0014)

[52 FR 8831, Mar. 19, 1987, as amended at 52 FR 23031, June 17, 1987. Redesignated at 53 FR 41523, Oct. 21, 1988]

PART 314—APPLICATIONS FOR FDA APPROVAL TO MARKET A NEW DRUG

Subpart A—General Provisions

Subpart B—Applications

Subpart C—Abbreviated Applications

Subpart D—FDA Action on Applications and Abbreviated Applications

Subpart E—Hearing Procedures for New Drugs

Subpart F—Administrative Procedures for Antibiotics

Subpart G—Miscellaneous Provisions

Subpart H—Accelerated Approval of New Drugs for Serious or Life-Threatening Illnesses

AUTHORITY: 21 U.S.C. 321, 331, 351, 352, 353, 355, 371, 374, 379e.

SOURCE: 50 FR 7493, Feb. 22, 1985, unless otherwise noted.

§ 50.20 General requirements for informed consent.

Except as provided in § 50.23, no investigator may involve a human being as a subject in research covered by these regulations unless the investigator has obtained the legally effective informed consent of the subject or the subject's legally authorized representative. An investigator shall seek such consent only under circumstances that provide the prospective subject or the representative sufficient opportunity to consider whether or not to participate and that minimize the possibility of coercion or undue influence. The information that is given to the subject or the representative shall be in language understandable to the subject or the representative. No informed consent, whether oral or written, may include any exculpatory language through which the subject or the representative is made to waive or appear to waive any of the subject's legal rights, or releases or appears to release the investigator, the sponsor, the institution, or its agents from liability for negligence.

§ 50.25 Elements of informed consent.

(a) *Basic elements of informed consent.* In seeking informed consent, the following information shall be provided to each subject:

(1) A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the subject's participation, a description of the procedures to be followed, and identification of any procedures which are experimental.

(2) A description of any reasonably foreseeable risks or discomforts to the subject.

(3) A description of any benefits to the subject or to others which may reasonably be expected from the research.

(4) A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject.

(5) A statement describing the extent, if any, to which confidentiality of records identifying the subject will be maintained and that notes the possibility that the Food and Drug Administration may inspect the records.

(6) For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of, or where further information may be obtained.

(7) An explanation of whom to contact for answers to pertinent questions about the research and research subjects' rights, and whom to contact in the event of a research-related injury to the subject.

(8) A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

(b) *Additional elements of informed consent.* When appropriate, one or more of the following elements of information shall also be provided to each subject:

(1) A statement that the particular treatment or procedure may involve risks to the subject (or to the embryo or fetus, if the subject is or may become pregnant) which are currently unforeseeable.

(2) Anticipated circumstances under which the subject's participation may be terminated by the investigator without regard to the subject's consent.

(3) Any additional costs to the subject that may result from participation in the research.

(4) The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject.

(5) A statement that significant new findings developed during the course of the research which may relate to the subject's willingness to continue participation will be provided to the subject.

(6) The approximate number of subjects involved in the study.

(c) The informed consent requirements in these regulations are not intended to preempt any applicable Federal, State, or local laws which require additional information to be disclosed for informed consent to be legally effective.

(d) Nothing in these regulations is intended to limit the authority of a physician to provide emergency medical care to the extent the physician is permitted to do so under applicable Federal, State, or local law.

Information Sheet for a Claim of Categorical Exclusion for an IND Under 21 CFR 25.24

For those wastes generated in the production and use of the product which will be controlled, please include documentation that such waste storage or disposal is in compliance with federal, state and local requirements for hazardous waste production. As an alternative, identify any generally recognized, scientifically sound control procedures which have been implemented to reduce the likelihood of inadvertent release of potentially toxic materials into the environment (e.g., compliance with the NIH Guidelines for Research Involving Recombinant DNA Molecules [51 FR 16958 (1986)] and/or compliance with the EPA Effluent Guidelines and Standards for Pharmaceutical Manufacturing [40 CFR 439]). If these alternatives are not applicable, a description of the control procedures actually used to prevent waste from entering the environment should be submitted.

For those wastes generated in the production and use of the product which will not be controlled, please list the potentially toxic waste compounds, including the quantities and concentrations which may be expected to enter the environment from both productions of the product and from the intended clinical studies, and briefly describe the immediate environment into which such release will occur. Further, provide the appropriate references or experimental data from which it may be reasonably concluded that such release is non-toxic.

If the waste to be generated during the production and proposed investigational use of this product is either not controlled or is not reasonably expected to be non-toxic in the environment to which it will be released, please submit an environmental assessment using the format described in 21 CFR 25.31.

If actions under proposed amendments to this IND substantially alter the quantity, quality or conditions of waste release in such a way as to alter the basis for either a claim of categorical exclusion or an environmental assessment, then such amendments should be supported by the appropriate data for a claim of categorical exclusion or an amended environmental assessment for wastes generated under the proposed amendments to this IND.

An investigator sponsored IND for which no additional product manufacturing is intended will ordinarily have addressed these environmental issues by incorporating the manufacturer's IND or MF by cross reference. However, if the use of the product during clinical investigation is expected to result in the uncontrolled release of toxic materials into the environment then an environmental assessment should be submitted.

3/30/94

Attachment J

Power of Attorney by Assignee

I hereby certify that this paper is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EM 021167715US on the date shown below in an envelope addressed to: Mail Stop Hatch-Waxman PTE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: August 2, 2012 Signature: (Shannon Reaney)

Docket No.: 146392015800
Client Ref. No.: P1467R2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Mark SLIWKOWSKI

Patent No.: 6,949,245

Issued: September 27, 2005

Application No: 09/602,812

For: HUMANIZED ANTI-ERBB2 ANTIBODIES AND TREATMENT WITH ANTI-ERBB2 ANTIBODIES – Application for § 156 Patent Term Extension

Attorney Docket No: 146392015800

Assignee: Genentech, Inc.

Unit: Office of Patent Legal Administration

POWER OF ATTORNEY

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Madam:

I hereby appoint Practitioners associated with the Customer Number 25226 as Genentech, Inc.'s attorney(s) or agent(s) in connection with the application for patent term extension under 35 U.S.C. § 156 for U.S. Patent No. 6,949,245, identified above, and to transact all business in the United States Patent and Trademark Office (USPTO) connected therewith. I authorize Practitioners associated with the Customer Number 25226 to file and prosecute the patent term extension application under 35 U.S.C. § 156 for U.S. Patent No. 6,949,245.

I understand that the Practitioners associated with the Customer Number 25226 will file and prosecute the Application as Genentech, Inc.'s representative, pursuant to 37 C.F.R. §1.730(b), and hereby grant the Practitioners associated with the Customer Number 25226 any authorizations from Genentech, Inc. necessary to act in this capacity.

Please direct all inquiries, questions, and communications regarding this application for term extension to the address associated with the above-mentioned Customer Number 25226.

The correspondence address for U.S. Patent No. 6,949,245 is unchanged for all other purposes.

Applicant represents that it is the assignee of the entire interest in and to United States Letters Patent No. 6,949,245, granted to Mark SLIWKOWSKI by virtue of an assignment of such patent to Genentech, Inc., recorded August 3, 2000, at Reel 011128, Frame 0088.

Dated July 30, 2012

Respectfully submitted,

By _____

Name: Timothy R. Schwartz, Ph.D

Title: Associate General Counsel
Genentech, Inc
Authorized Corporate Signatory

Phone: 650-225-7467

Attachment K

Petition Decision for U.S. Patent No. 6,949,245

UNITED STATES PATENT AND TRADEMARK OFFICE

COMMISSIONER FOR PATENTS
UNITED STATES PATENT AND TRADEMARK OFFICE
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450
www.uspto.gov

Paper No.

Genentech Inc
Attn Wendy Lee
1 DNA Way
San Francisco, CA 94080-4990

In re Patent No. 6,949,245	:	DECISION ON REQUEST FOR
Mark Sliwkowski	:	RECONSIDERATION OF
Issue Date: September 27, 2005	:	PATENT TERM ADJUSTMENT
Application No. 09/602,812	:	and
Filed: June 23, 2000	:	NOTICE OF INTENT TO ISSUE
Attorney Docket No. P1467R2	:	CERTIFICATE OF CORRECTION
	:	

This is a decision on the "REQUEST FOR RECONSIDERATION OF REVISION OF PATENT TERM ADJUSTMENT UNDER 37 C.F.R. § 1.705(d)," filed December 5, 2005, with a certificate of mailing dated November 28, 2005. Patentee requests that the patent term adjustment indicated on the patent be corrected from two hundred fifteen (215) days to three hundred thirty-five (335) days.

The request for reconsideration of patent term adjustment is **GRANTED**.

The patent term adjustment indicated in the patent is to be corrected by issuance of a certificate of correction showing a revised Patent Term Adjustment of **THREE HUNDRED THIRTY-FIVE (335)** days.

On September 27, 2005, the application matured into U.S. Patent No. 6,949,245, with a revised patent term adjustment of 215 days. By virtue of the certificate of mailing dated Monday, November 28, 2005, patentee timely submitted this request for reconsideration of patent term adjustment (with required fee), asserting that the correct number of days of Patent Term Adjustment is 335 days. Patentee asserts that the 120-day applicant delay associated with the "miscellaneous paper" filed on April 12, 2005, was recorded in error. Patentee states that

the only papers filed on April 12, 2005 were an application for patent term adjustment including an exhibit containing a copy of an earlier filed Information Disclosure Statement and a petition under § 1.183 requesting waiver of the requirement that the application for patent term adjustment be filed before the payment of the issue fee.

37 C.F.R. § 1.704(e) provides that:

> Submission of an application for patent term adjustment under § 1.705(b) (with or without request under § 1.705(c) for reinstatement of reduced patent term adjustment) will not be considered a failure to engage in reasonable efforts to conclude prosecution (processing or examination) of the application under paragraph (c)(10) of this section.

A review of the record confirms that the reduction of 120 days is based on the filing of the application for patent term adjustment, which included an exhibit and a petition for waiver of the timeliness requirement. In view thereof, the reduction of 120 days was not warranted.

The patent should have issued with a revised patent term adjustment of three hundred thirty-five (335) days.

The Office acknowledges submission of the $200.00 fee set forth in 37 CFR 1.18(e). No additional fees are required.

The application is being forwarded to the Certificates of Correction Branch for issuance of a certificate of correction in order to rectify this error. The certificate of correction will indicate that the term of the above-identified patent is extended or adjusted by **THREE HUNDRED THIRTY-FIVE (335)** days.

Telephone inquiries specific to this matter should be directed to the undersigned at (571) 272-3219.

Nancy Johnson
Senior Petitions Attorney
Office of Petitions

Enclosure: Copy of DRAFT Certificate of Correction

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT : 6,949,245 B1

DATED : September 27, 2005

INVENTOR(S) : Sliwkowski

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the cover page,

[*] Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 USC 154(b) by (215) days

Delete the phrase "by 215" and insert – by 335 days--